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Amniocentesis and chorionic villus sampling for prenatal diagnosis (Review)

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[Intervention Review]

Amniocentesis and chorionic villus sampling for prenatal diagnosis

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ABSTRACT

Background

During pregnancy, fetal cells suitable for genetic testing can be obtained from amniotic fluid by amniocentesis (AC), placental tissue by chorionic villus sampling (CVS), or fetal blood. A major disadvantage of second trimester amniocentesis is that the results are available relatively late in pregnancy (after 16 weeks' gestation). Earlier alternatives are chorionic villus sampling (CVS) and early amniocentesis, which can be performed in the first trimester of pregnancy.

Objectives

The objective of this review was to compare the safety and accuracy of all types of AC (i.e. early and late) and CVS (e.g. transabdominal, transcervical) for prenatal diagnosis.

Search methods

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (3 March 2017), ClinicalTrials.gov, the WHO International Clinical Trials Registry Platform (ICTRP; 3 March 2017), and reference lists of retrieved studies.

Selection criteria

All randomised trials comparing AC and CVS by either transabdominal or transcervical route.

Data collection and analysis

Two review authors independently assessed trials for inclusion and risk of bias, extracted data and checked them for accuracy. The quality of the evidence was assessed using the GRADE approach.

Main results

We included a total of 16 randomised studies, with a total of 33,555 women, 14 of which were deemed to be at low risk of bias. The number of women included in the trials ranged from 223 to 4606.

Studies were categorized into six comparisons: 1. second trimester AC versus control; 2. early versus second trimester AC; 3. CVS versus second trimester AC; 4. CVS methods; 5. Early AC versus CVS; and 6. AC with or without ultrasound.

One study compared second trimester AC with no AC (control) in a low risk population (women = 4606). Background pregnancy loss was around 2%. Second trimester AC compared to no testing increased total pregnancy loss by another 1%. The confidence intervals (CI) around this excess risk were relatively large (3.2% versus 2.3 %, average risk ratio (RR) 1.41, 95% CI 0.99 to 2.00; moderate-quality evidence). In the

same study, spontaneous miscarriages were also higher (2.1% versus 1.3%; average RR 1.60, 95% CI 1.02 to 2.52; high-quality evidence). The number of congenital anomalies was similar in both groups (2.0% versus 2.2%, average RR 0.93, 95% CI 0.62 to 1.39; moderate-quality evidence).

One study (women = 4334) found that early amniocentesis was not a safe early alternative compared to second trimester amniocentesis because of increased total pregnancy losses (7.6% versus 5.9%; average RR 1.29, 95% CI 1.03 to 1.61; high-quality evidence), spontaneous miscarriages (3.6% versus 2.5%, average RR 1.41, 95% CI 1.00 to 1.98; moderate-quality evidence), and a higher incidence of congential anomalies, including talipes (4.7% versus 2.7%; average RR 1.73, 95% CI 1.26 to 2.38; high-quality evidence).

When pregnancy loss after CVS was compared with second trimester AC, there was a clinically significant heterogeneity in the size and direction of the effect depending on the technique used (transabdominal or transcervical), therefore, the results were not pooled. Only one study compared transabdominal CVS with second trimester AC (women = 2234). They found no clear difference between the two procedures in the total pregnancy loss (6.3% versus 7%; average RR 0.90, 95% CI 0.66 to 1.23, low-quality evidence), spontaneous miscarriages (3.0% versus 3.9%; average RR 0.77, 95% CI 0.49 to 1.21; low-quality evidence), and perinatal deaths (0.7% versus 0.6%; average RR 1.18, 95% CI 0.40 to 3.51; low-quality evidence). Transcervical CVS may carry a higher risk of pregnancy loss (14.5% versus 11.5%; average RR 1.40, 95% CI 1.09 to 1.81), but the results were quite heterogeneous.

Five studies compared transabdominal and transcervical CVS (women = 7978). There were no clear differences between the two methods in pregnancy losses (average RR 1.16, 95% CI 0.81 to 1.65; very low-quality evidence), spontaneous miscarriages (average RR 1.68, 95% CI 0.79 to 3.58; very low-quality evidence), or anomalies (average RR 0.68, 95% CI 0.41 to 1.12; low-quality evidence). We downgraded the quality of the evidence to low due to heterogeneity between studies. Transcervical CVS may be more technically demanding than transabdominal CVS, with more failures to obtain sample (2.0% versus 1.1%; average RR 1.79, 95% CI 1.13 to 2.82, moderate-quality evidence).

Overall, we found low-quality evidence for outcomes when early amniocentesis was compared to transabdominal CVS. Spontaneous miscarriage was the only outcome supported by moderate-quality evidence, resulting in more miscarriages after early AC compared with transabdominal CVS (2.3% versus 1.3%; average RR 1.73, 95% CI 1.15 to 2.60). There were no clear differences in pregnancy losses (average RR 1.15, 95% CI 0.86 to 1.54; low-quality evidence), or anomalies (average RR 1.14, 95% CI 0.57 to 2.30; very low-quality evidence).

We found one study that examined AC with or without ultrasound, which evaluated a type of ultrasound-assisted procedure that is now considered obsolete.

Authors' conclusions

Second trimester amniocentesis increased the risk of pregnancy loss, but it was not possible to quantify this increase precisely from only one study, carried out more than 30 years ago.

Early amniocentesis was not as safe as second trimester amniocentesis, illustrated by increased pregnancy loss and congenital anomalies (talipes). Transcervical chorionic villus sampling compared with second trimester amniocentesis may be associated with a higher risk of pregnancy loss, but results were quite heterogeneous.

Diagnostic accuracy of different methods could not be assessed adequately because of incomplete karyotype data in most studies.

PLAIN LANGUAGE SUMMARY

Amniocentesis and placental sampling for pre-birth diagnosis

What is the issue?

Many women want to be reassured that their unborn baby is healthy. Second trimester amniocentesis performed around 16 weeks' gestation is the test most often used. A needle is inserted through the abdomen into the uterus to remove a sample of amniotic fluid. Early amniocentesis can be done before 15 weeks. With chorionic villus sampling, a needle is used to withdraw a sample of placental tissue. The needle can be inserted through the abdomen (transabdominal), or vaginally through the cervix (transcervical).

Why is this important?

It is important that tests used to indicate high-risk (screening tests), and tests used to make a diagnosis (diagnostic tests) are safe and accurate. It is also important that diagnostic tests can be done early enough to allow parents the choice of early termination of pregnancy.

What evidence did we find?

We searched for evidence on 3 March 2017; we included 16 randomised controlled trials in the review, with a total of 33,555 women. The overall risk of bias was low, with very low to high-quality evidence supporting the outcomes studied. One study of 4606 women found that a second trimester amniocentesis increased spontaneous miscarriages and pregnancy losses, but the estimate remains quite imprecise, ranging from 0 to 2%.

Early amniocentesis was not as safe as second trimester amniocentesis because of increased pregnancy loss and spontaneous miscarriages, and higher occurrences of anomalies, particularly deformed or clubfeet (talipes).

Low-quality evidence found no clear differences in pregnancy loss or spontaneous miscarriages after transabdominal chorionic villus sampling or second trimester amniocentesis. Transcervical chorionic villus sampling may increase the total risk of pregnancy loss compared with a second trimester amniocentesis, mostly because of increased spontaneous miscarriages. Healthcare staff may have found transcervical chorionic villus sampling more difficult to perform than transabdominal chorionic villus sampling, because there were more failures to obtain a sample, and more repeat testing.

What does this mean?

High-quality evidence supported second trimester amniocentesis as the procedure of first choice for testing from 15 weeks' gestation or later. When a test is required earlier than 15 weeks' gestation, low-quality to moderate-quality evidence suggested that transabdominal chorionic villus sampling could be considered the procedure of first choice, depending on the outcome of interest.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Second trimester amniocentesis compared to control for prenatal diagnosis

Second trimester amniocentesis compared to control for prenatal diagnosis						
Patient or population: prenatal diagnosis **Setting:** hospitals in Denmark **Intervention:** second trimester amniocentesis **Comparison:** control						
Outcomes	**Anticipated absolute effects* (95% CI)**		**Relative effect (95% CI)**	**№ of participants (studies)**	**Quality of the evidence (GRADE)**	**Comments**
	Risk with control	**Risk with second trimester amniocentesis (AC)**				
All known pregnancy loss (including termination of pregnancy)	Study population 23 per 1000	32 per 1000 (22 to 45)	RR 1.41 (0.99 to 2.00)	4606 (1 RCT)	⊕⊕⊕⊖ MODERATE[1]	
Spontaneous miscarriage	Study population 13 per 1000	21 per 1000 (13 to 33)	RR 1.60 (1.02 to 2.52)	4606 (1 RCT)	⊕⊕⊕⊕ HIGH	
Sampling failure	-	-	-	-	-	No trial reported this outcome
Laboratory failure	Study population 0 per 1000	0 per 1000 (0 to 0)	RR 27.02 (1.61 to 454.31)	4606 (1 RCT)	⊕⊕⊕⊕ HIGH	There were no events in the control group and only 13 in the AC group, so it was not possible to calculate the anticipated absolute effect.
Known false negative after birth	-	-	-	-	-	No trial reported this outcome
Delivery before 33 weeks	-	-	-	-	-	No trial reported this outcome
Anomalies (all recorded)	Study population 22 per 1000	20 per 1000 (13 to 30)	RR 0.93 (0.62 to 1.39)	4507 (1 RCT)	⊕⊕⊕⊖ MODERATE [1]	

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Wide confidence intervals crossing the line of no effect (-1)

Summary of findings 2. Early compared to second trimester amniocentesis for prenatal diagnosis

Early compared to second trimester amniocentesis for prenatal diagnosis

Patient or population: prenatal diagnosis
Setting: hospitals in Canada
Intervention: early amniocentesis
Comparison: second trimester amniocentesis

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with second trimester amniocentesis (AC)	Risk with early AC				
All known pregnancy loss (including termination of pregnancy)	Study population		RR 1.29 (1.03 to 1.61)	4334 (1 RCT)	⊕⊕⊕⊕ HIGH [1]	
	59 per 1000	76 per 1000 (61 to 95)				
Spontaneous miscarriage	Study population		RR 1.41 (1.00 to 1.98)	4334 (1 RCT)	⊕⊕⊕⊖ MODERATE [1, 2]	
	25 per 1000	36 per 1000 (25 to 50)				
Sampling failure	Study population		RR 4.53 (0.53 to 38.56)	629 (1 RCT)	⊕⊕⊕⊖ MODERATE [1, 2]	
	3 per 1000	15 per 1000				

		(2 to 129)				
Laboratory failure	Study population		RR 9.76 (3.49 to 27.26)	4368 (1 RCT)	⊕⊕⊕⊕ HIGH [1]	
	2 per 1000	18 per 1000 (6 to 50)				
Known false negative after birth	Study population		RR 3.00 (0.12 to 73.67)	4368 (1 RCT)	⊕⊕⊕⊖ MODERATE [1, 2]	There were no events in the 2nd trimester AC group and only one in the early AC group, so it was not possible to calculate the anticipated absolute effect
	0 per 1000	0 per 1000 (0 to 0)				
Known false negative after birth - Incorrect sex determination	Study population		RR 5.00 (0.24 to 104.18)	4368 (1 RCT)	⊕⊕⊕⊖ MODERATE [1, 2]	There were no events in the 2nd trimester AC group and only 2 in the early AC group, so it was not possible to calculate the anticipated absolute effect.
	0 per 1000	0 per 1000 (0 to 0)				
Delivery before 33 weeks	-	-	-	-		No trial reported this outcome
Anomalies (all recorded)	Study population		RR 1.73 (1.26 to 2.38)	4334 (1 RCT)	⊕⊕⊕⊕ HIGH [2]	
	27 per 1000	46 per 1000 (34 to 64)				

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] One study, contributing 100% data, had unclear allocation concealment for one trial site, and satisfactory concealment for the other trial site (not downgraded)
2 Wide 95% confidence interval, crossing the line of no effect (-1)

Summary of findings 3. Transabdominal chorionic villus sampling compared to second trimester amniocentesis for prenatal diagnosis

Chorionic villus sampling compared to second trimester amniocentesis for prenatal diagnosis

Patient or population: prenatal diagnosis
Setting: hospital in Denmark
Intervention: chorionic villus sampling
Comparison: second trimester amniocentesis

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with second trimester amniocentesis	Risk with chorionic villus sampling				
All known pregnancy loss (including termination of pregnancy)	Study population		RR 0.90 (0.66 to 1.23)	2234 (1 RCT)	⊕⊕⊖⊖ LOW[1, 2]	
	70 per 1000	63 per 1000 (46 to 86)				
Spontaneous miscarriage	Study population		RR 0.77 (0.49 to 1.21)	2069 (1 RCT)	⊕⊕⊖⊖ LOW[1, 2]	
	39 per 1000	30 per 1000 (19 to 47)				
Sampling failure	-	-	-	-	-	No trial reported this outcome
Laboratory failure	-	-	-	-	-	No trial reported this outcome
Known false negative after birth	-	-	-	-	-	No trial reported this outcome
Delivery before 33 weeks	-	-	-	-	-	No trial reported this outcome
Perinatal deaths (stillbirths and neonatal deaths in the first week of life)	Study population		RR 1.18 (0.40 to 3.51)	2069 (1 RCT)	⊕⊕⊖⊖ LOW[1, 2]	
	6 per 1000	7 per 1000 (2 to 21)				

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Wide 95% confidence intervals that cross the line of no effect (-1)
[2] One study, contributing 100% data, with unclear method of randomisation (-1)

Summary of findings 4. Transcervical compared to transabdominal chorionic villus sampling for prenatal diagnosis

Transcervical compared to transabdominal chorionic villus samplingfor prenatal diagnosis

Patient or population: prenatal diagnosis
Setting: Denmark, Italy, United States
Intervention: transcervical chorionic villus sampling
Comparison: transabdominal chorionic villus sampling

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with transabdominal chorionic villus sampling	Risk with transcervical chorionic villus sampling				
All known pregnancy loss (including termination of pregnancy)	Study population		RR 1.16 (0.81 to 1.65)	7978 (5 RCTs)	⊕⊖⊖⊖ VERY LOW[1, 2, 3]	
	74 per 1000	86 per 1000 (60 to 123)				
Spontaneous miscarriage	Study population		RR 1.68 (0.79 to 3.58)	3384 (4 RCTs)	⊕⊖⊖⊖ VERY LOW [1, 2, 4]	
	45 per 1000	76 per 1000 (36 to 162)				
Sampling failure	Study population		RR 1.79 (1.13 to 2.82)	5231 (4 RCTs)	⊕⊕⊕⊖ MODERATE [4]	
	11 per 1000	20 per 1000 (12 to 31)				

Laboratory failure	Study population		RR 2.23 (0.69 to 7.22)	1194 (1 RCT)	⊕⊕⊝⊝ LOW [1, 5]	
	7 per 1000	15 per 1000 (5 to 49)				
Known false negative after birth	-	-	-	-	-	No trial reported this outcome
Delivery before 33 weeks	-	-	-	-	-	No trial reported this outcome
Anomalies (all recorded)	Study population		RR 0.68 (0.41 to 1.12)	3622 (2 RCTs)	⊕⊕⊝⊝ LOW [1, 6]	
	20 per 1000	14 per 1000 (8 to 22)				

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Wide 95% confidence intervals crossing the line of no effect (-1)
[2] Statistical heterogeneity I^2 > 60%
[3] Four of five contributing studies did not specify randomisation method. All studies had design limitations. In one study, the proportion of cases where the operator deviated from the allocated procedure increased during the study (-1)
[4] Three of four studies, contributing > 95% weight, did not specify randomisation method. All studies had design limitations. In one study, the proportion of cases where the operator deviated from the allocated procedure increased during the study (-1).
[5] One study contributing data had design limitations - the proportion of cases where the operator deviated from the allocated procedure increased during the study (-1).
[6] One of two contributing studies did not specify randomisation method. All studies had design limitations. In one study, the proportion of cases where the operator deviated from the allocated procedure increased during the study (-1).

Summary of findings 5. Early amniocentesis compared to transabdominal chorionic villus sampling for prenatal diagnosis

Early amniocentesis compared to transabdominal chorionic villus samplingfor prenatal diagnosis

Patient or population: prenatal diagnosis
Setting: Canada, Denmark, the Netherlands, United Kingdom, United States
Intervention: early amniocentesis
Comparison: transabdominal chorionic villus sampling

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with transabdominal chorionic villus sampling	Risk with early amniocentesis				
All known pregnancy loss (including termination of pregnancy)	Study population		RR 1.15 (0.86 to 1.54)	5491 (4 RCTs)	⊕⊕⊝⊝ LOW [1, 2]	
	30 per 1000	35 per 1000 (26 to 47)				
Spontaneous miscarriage	Study population		RR 1.73 (1.15 to 2.60)	5491 (4 RCTs)	⊕⊕⊕⊝ MODERATE [1]	
	13 per 1000	23 per 1000 (15 to 34)				
Sampling failure	Study population		RR 0.58 (0.24 to 1.38)	5566 (4 RCTs)	⊕⊕⊝⊝ LOW [1, 2]	
	8 per 1000	5 per 1000 (2 to 11)				
Laboratory failure	Study population		RR 0.74 (0.34 to 1.63)	5566 (4 RCTs)	⊕⊕⊝⊝ LOW [1, 2]	
	6 per 1000	4 per 1000 (2 to 9)				
Known false negative after birth	Study population		Not estimable	555 (1 RCT)	⊕⊕⊝⊝ LOW [3, 4]	
	0 per 1000	0 per 1000 (0 to 0)				
Delivery before 33 weeks	Study population		RR 0.50 (0.09 to 2.73)	1121 (1 RCT)	⊕⊕⊝⊝ LOW [2, 3]	
	7 per 1000	4 per 1000 (1 to 19)				

Anomalies (all recorded)	Study population		RR 1.14 (0.57 to 2.30)	5305 (4 RCTs)	⊕⊖⊖⊖ VERY LOW [1, 2, 5]
	25 per 1000	29 per 1000 (14 to 58)			

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Three of four studies, contributing > 50% weight of analysis, did not specify randomisation method (-1).
[2] Wide 95% confidence intervals crossing the line of no effect (-1)
[3] Randomisation method not specified (-1)
[4] One study with 555 women and no events therefore not possible to estimate risk (-1)
[5] Statistical heterogeneity I^2>60% (-1)

BACKGROUND

Description of the condition

Most women wish to be reassured that their unborn baby is healthy. Inevitably, any screening programme that aims to provide such reassurance will cause anxiety while waiting for the test results. The additional problems are 'false positive' screening test results (maternal serum screening and ultrasound) and lack of therapeutic options for chromosomal abnormalities. Therefore, the aim is to select screening and diagnostic tests that are both accurate and safe, and can be done early in pregnancy to allow the choice of termination of pregnancy.

Ultrasound is the method of choice for detection of anatomical problems (e.g. absent kidneys, spina bifida), but provides no information on the genetic constitution of a fetus. Maternal serum screening, alone or in combination with ultrasound, is often used to identify fetuses at risk of Down syndrome, but the definitive chromosomal diagnosis can only be made from fetal cells.

Description of the intervention

Fetal cells suitable for genetic testing could be obtained from maternal blood or pre-implantation embryos. However, the former test is still being developed, while the latter requires in vitro fertilisation, which is often not feasible. At present, only the analysis of fetal cells from amniotic fluid, placenta (chorionic villus tissue), or fetal blood can result in an accurate prenatal diagnosis.

Second trimester amniocentesis, a needle puncture through the overlying skin into the uterus and amniotic cavity, followed by aspiration of amniotic fluid, is traditionally performed around 16 weeks' gestation. Observational data from the 1970s suggested that, at this gestation, relatively large amounts of amniotic fluid (up to 20 mL) could be aspirated without significant technical difficulties. This amount of amniotic fluid was needed to yield a sufficient number of viable fetal cells to minimise the risk of laboratory failure. In 1977, the MRC Canadian Study reported a rate of successful culture of only 82% if obtained before 15 weeks, compared to 94% when obtained at 16 weeks or later. Another disincentive to perform earlier sampling was a belief that aspiration of large amounts of amniotic fluid earlier in gestation would be more likely to cause neonatal orthopaedic (talipes) and respiratory complications (respiratory distress syndrome).

A major disadvantage of second trimester amniocentesis is that a final result is usually available only after 17 weeks' gestation. Such a long waiting period for a diagnosis can be very distressing for couples, particularly when most obstetricians are reluctant to offer a surgical termination late in pregnancy. Earlier options include chorionic villus sampling (CVS) and early amniocentesis.

Chorionic villus sampling was first described in China in the mid-1970s, and developed further in the Western world during the 1980s (China 1975). The procedure involves aspiration of placental tissue rather than amniotic fluid. Ultrasound guided aspiration can be performed using either percutaneous transabdominal, the transvaginal, or transcervical approach. Currently, the choice of the approach and the choice of instruments tend to be based upon the operator's personal preference (Alfirevic 2002).

How the intervention might work

There is an understandable desire to perform CVS as early as possible. Technically, this can be done successfully as early as six weeks' gestation. However, a few clusters of limb reduction defects have been reported following CVS, with a trend toward an increased incidence of these defects when CVS was done before nine weeks' gestation (for review of the evidence, see Jackson 1993). Subsequent, large epidemiological follow-up studies failed to confirm this association (Froster 1996), but most clinicians delay this procedure until after 10 weeks' gestation.

Early amniocentesis (9 to 14 weeks' gestation) was introduced in the late 1980s. Technically, it is the same as a 'late' procedure, except that less amniotic fluid is removed. Ultrasound needle guidance is considered to be an essential part of the procedure because of the relatively small target area. The presence of two separate membranes (amnion and chorion) until 15 weeks' gestation creates an additional technical difficulty. Only the amniotic (inner) sac should be aspirated, because the outer sac does not contain sufficient numbers of living fetal cells.

Why it is important to do this review

With a development of a new non-invasive prenatal diagnostic test, such as fetal cell free DNA testing from maternal blood, and recent reports of observational studies that emphasise the 'low' risk of invasive testing, it is important to critically appraise the data from randomised controlled trials for different invasive procedures. The results will help to formulate the heath care policies and clinical decisions of the future.

OBJECTIVES

The objective of this review was to compare the safety and accuracy of all types of amniocentesis (i.e. early and late) and chorionic villus sampling (e.g. transabdominal, transcervical) for prenatal diagnosis.

METHODS

Criteria for considering studies for this review

Types of studies

We included all randomised comparisons of late amniocentesis (after 15 weeks' gestation), early amniocentesis (before 15 weeks' gestation), and chorionic villus sampling (either transabdominally or transvaginally) with each other, or with no testing. We excluded cluster-randomised trials and quasi-randomised studies (e.g. alternate allocation).

Types of participants

Pregnant women requesting invasive prenatal diagnostic testing for fetal chromosomal or genetic disorders.

Types of interventions

- Second trimester amniocentesis (after 15 completed weeks of gestation).
- Early amniocentesis (before 15 completed weeks of gestation (i.e. 14 weeks and 6 days or less)).
- Transabdominal, transcervical, or transvaginal chorionic villus sampling.

Types of outcome measures

All the outcomes of interest were divided into the following groups.

Primary outcomes

(i) Pregnancy outcome

- All known pregnancy losses (including termination of pregnancy).
- Spontaneous miscarriage (pregnancy loss before viability - usually before 24 weeks of pregnancy).
- Spontaneous miscarriage after test (pregnancy loss in women who actually had the test performed).

Secondary outcomes

(ii) Outcomes related to technical difficulties in sampling

- Non-compliance with allocated procedure.
- Sampling failure.
- Multiple insertions.
- Second test performed.

(iii) Outcomes related to cytogenetic analysis

- Laboratory failure.
- All non-mosaic abnormalities.
- All mosaics (karyotypes with two or more cell lines).
- True mosaics.
- Confined mosaics (two or more cell lines present in the placenta but not in the fetus).
- Maternal contamination.
- Known false positive after birth.
- Known false negative after birth.
- Reporting time (interval between sampling and result).

(iv) Pregnancy complications

- Vaginal bleeding after test.
- Amniotic leakage after test.
- Vaginal bleeding after 20 weeks.
- Pre-labour ruptured membranes before 28 weeks.
- Antenatal hospital admission.
- Delivery before 37 weeks.
- Delivery before 33 weeks.

(v) Pregnancy outcome

- Termination of pregnancy (all).
- Perinatal mortality (stillbirths and neonatal deaths in the first week of life).
- Stillbirths.
- Neonatal death (death in the first week of life).
- All recorded deaths after viability.

(vi) Neonatal complications

- Anomalies (all recorded).
- Talipas (clubfoot).
- Talipes equinovarus (the foot is plantar flexed, inverted, and markedly adducted).
- Haemangiomas (localised vascular lesions of the skin and subcutaneous tissue).
- Limb reduction defects.
- Admission to special care baby unit.
- Neonatal respiratory distress syndrome (defined by authors).
- Birthweight below the 10th centile.
- Birthweight below the 5th centile.

While we sought all the above outcomes, we only included those with data in the analysis table. The data that were not prespecified by the review authors, but reported by the authors, were clearly labelled as such ('not prespecified'):

- Results given in less than 7 days (not pre-specified)
- Results given in less than 14 days (not pre-specified)
- Results given in less than 21 days (not pre-specified)
- Results given after 21 days (not pre-specified)
- Not wanting another baby at 22 weeks' gestation (not pre-specified)

Search methods for identification of studies

The following methods section of this review was based on a standard template used by Cochrane Pregnancy and Childbirth.

Electronic searches

We searched Cochrane Pregnancy and Childbirth's Trials Register by contacting their Information Specialist (3 March 2017).

The Register is a database containing over 23,000 reports of controlled trials in the field of pregnancy and childbirth. For full search methods used to populate Pregnancy and Childbirth's Trials Register including the detailed search strategies for CENTRAL, MEDLINE, Embase and CINAHL; the list of handsearched journals and conference proceedings, and the list of journals reviewed via the current awareness service, please follow this link to the editorial information about the Cochrane Pregnancy and Childbirth in the Cochrane Library and select the '***Specialized Register***' section from the options on the left side of the screen.

Briefly, Cochrane Pregnancy and Childbirth's Trials Register is maintained by their Information Specialist and contains trials identified from:

1. monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
2. weekly searches of MEDLINE (Ovid);
3. weekly searches of Embase (Ovid);
4. monthly searches of CINAHL (EBSCO);
5. handsearches of 30 journals and the proceedings of major conferences;
6. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Search results are screened by two people and the full text of all relevant trial reports identified through the searching activities described above is reviewed. Based on the intervention described, each trial report is assigned a number that corresponds to a specific Pregnancy and Childbirth review topic (or topics), and is then added to the Register. The Information Specialist searches the Register for each review using this topic number rather than

keywords. This results in a more specific search set which has been fully accounted for in the relevant review sections (Included studies; Excluded studies).

In addition, we searched ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform (ICTRP) for unpublished, planned and ongoing trial reports (3 March 2017) using the search terms detailed in Appendix 1.

Searching other resources

We searched the reference lists of retrieved studies.

We did not apply any language or date restrictions.

Data collection and analysis

For methods used in the previous version of this review, *see* Alfirevic 2003.

For this update, the following methods were used to assess the four reports that were identified in the updated search.

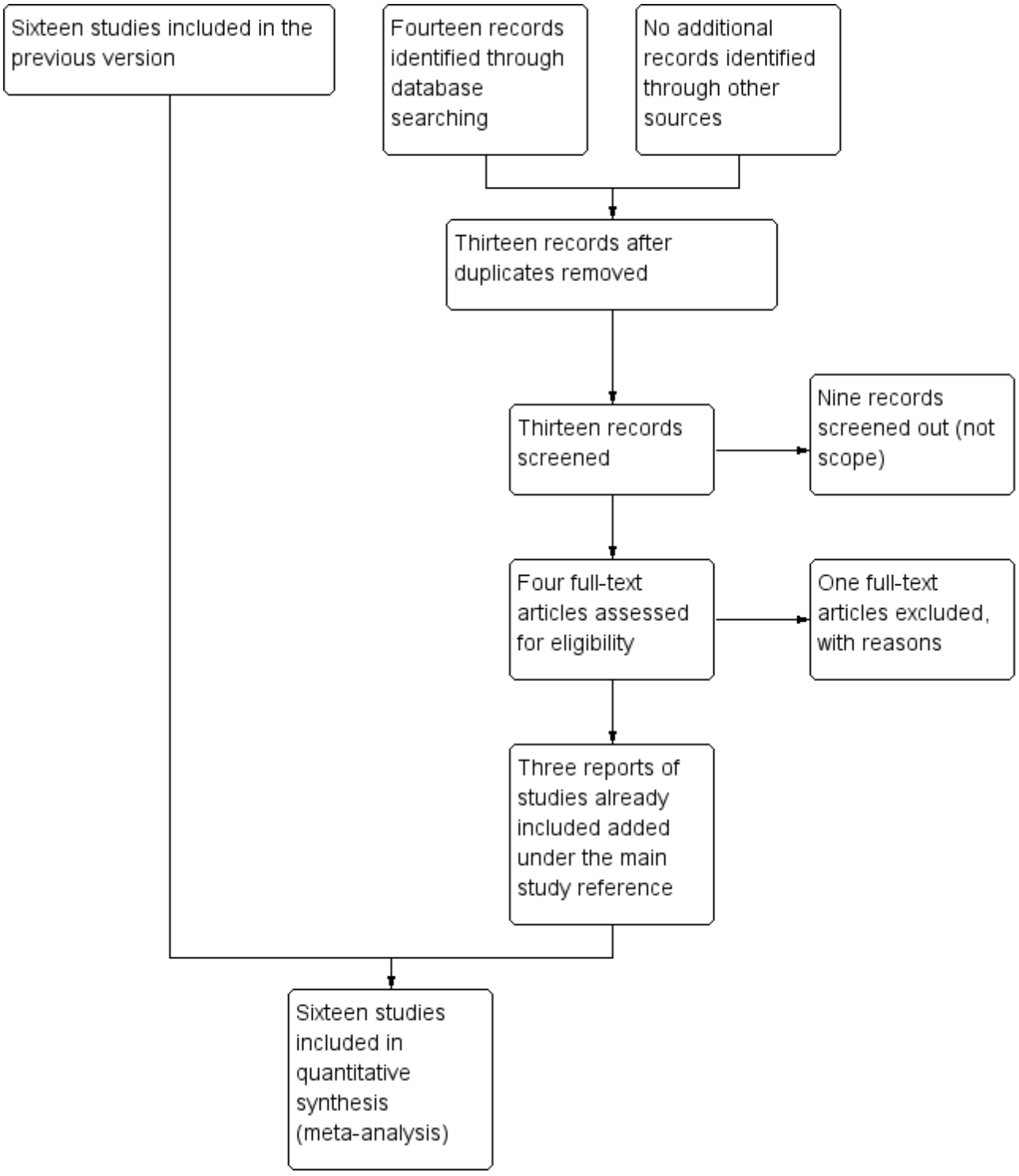
The following methods section of this review was based on a standard template used by Cochrane Pregnancy and Childbirth.

Selection of studies

Two review authors independently assessed for inclusion all the potential studies identified with the search strategy. We resolved any disagreement through discussion, or if required, we consulted the third review author.

We created a study flow diagram to map out the number of records identified, included, and excluded (Figure 1).

Figure 1. Study flow diagram.



Data extraction and management

We designed a form to extract data. For eligible studies, two review authors independently extracted the data using the agreed form. We resolved discrepancies through discussion, or if required, we consulted the third review author. Data were entered into Review Manager 5 software and checked for accuracy (RevMan 2014).

When information regarding any of the above was unclear, we planned to contact authors of the original reports to provide further details.

Assessment of risk of bias in included studies

Two review authors independently assessed risk of bias for each study using the criteria outlined in the *Cochrane Handbook*

for Systematic Reviews of Interventions (Higgins 2011). Any disagreement was resolved by discussion, or by involving a third assessor.

(1) Random sequence generation (checking for possible selection bias)

For each included study, we described the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.

We assessed the method as:

- low risk of bias (any truly random process, e.g. random number table, computer random number generator);
- high risk of bias (any non-random process, e.g. odd or even date of birth, hospital or clinic record number);
- unclear risk of bias.

(2) Allocation concealment (checking for possible selection bias)

For each included study, we described the method used to conceal allocation to interventions prior to assignment, and assessed whether the intervention allocation could have been foreseen in advance of, or during recruitment, or changed after assignment.

We assessed the methods as:

- low risk of bias (e.g. telephone or central randomisation, consecutively numbered sealed opaque envelopes);
- high risk of bias (open random allocation, unsealed or non-opaque envelopes, alternation, date of birth);
- unclear risk of bias.

(3.1) Blinding of participants and personnel (checking for possible performance bias)

For each included study, we described the methods used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. We considered that studies were at low risk of bias if they were blinded, or if we judged that the lack of blinding was unlikely to affect results. We assessed blinding separately for different outcomes or classes of outcomes.

We assessed the methods as:

- low, high, or unclear risk of bias for participants;
- low, high, or unclear risk of bias for personnel.

(3.2) Blinding of outcome assessment (checking for possible detection bias)

For each included study, we described the methods used, if any, to blind outcome assessors from the knowledge of which intervention a participant received. We assessed blinding separately for different outcomes or classes of outcomes.

We assessed methods used to blind outcome assessment as:

- low, high, or unclear risk of bias.

(4) Incomplete outcome data (checking for possible attrition bias due to the amount, nature and handling of incomplete outcome data)

For each included study, and for each outcome or class of outcomes, we described the completeness of data, including attrition, and exclusions from the analysis. We stated whether attrition and exclusions were reported, and the numbers included in the analysis at each stage (compared with the total randomised participants); reasons for attrition or exclusion where reported, and whether missing data were balanced across groups, or were related to outcomes. Where sufficient information was reported, or could be supplied by the trial authors, we planned to re-include missing data in the analyses that we undertook.

We assessed methods as:

- low risk of bias (e.g. no missing outcome data, missing outcome data balanced across groups);
- high risk of bias (e.g. numbers or reasons for missing data imbalanced across groups, 'as treated' analysis done with substantial departure of intervention received from that assigned at randomisation);
- unclear risk of bias.

(5) Selective reporting (checking for reporting bias)

For each included study, we described how we investigated the possibility of selective outcome reporting bias, and what we found.

We assessed the methods as:

- low risk of bias (where it was clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review had been reported);
- high risk of bias (where not all the study's pre-specified outcomes were reported, one or more reported primary outcomes were not pre-specified, outcomes of interest were reported incompletely, and so could not be used, study failed to include results of a key outcome that would have been expected to have been reported);
- unclear risk of bias.

(6) Other bias (checking for bias due to problems not covered by (1) to (5) above)

For each included study, we described any important concerns we had about other possible sources of bias.

(7) Overall risk of bias

We made explicit judgements about whether studies were at high risk of bias, according to the criteria given in the *Cochrane Handbook of Systematic Reviews of Interventions* (Higgins 2011). With reference to (1) to (6) above, we planned to assess the likely magnitude and direction of the bias and whether we considered it was likely to impact on the findings. In future updates, assuming we have sufficient studies, we will explore the impact of the level of bias through undertaking sensitivity analyses - *see* Sensitivity analysis.

Assessment of the quality of the evidence in included studies

For this update, we assessed the quality of the evidence using the GRADE approach, as outlined in the *GRADE Handbook (* Schünemann 2013). We assessed the quality of the body of

evidence relating to the following essential outcomes for decision making for the main comparisons: second trimester amniocentesis compared to control, early compared to second trimester amniocentesis, transcervical compared to transabdominal CVS, and early amniocentesis compared to transabdominal CVS:

1. All known pregnancy losses
2. Spontaneous miscarriage (pregnancy loss before viability - usually before 24 weeks of pregnancy)
3. Sampling failure
4. Laboratory failure
5. Known false negative after birth
6. Delivery before 33 weeks
7. Anomalies (all recorded)

For the subgroup comparison transabdominal CVS compared to second trimester amniocentesis, we assessed the evidence for the above outcomes using the GRADE approach, but exchanged 'anomalies' for 'perinatal death'.

We used GRADEpro GDT to import data from Review Manager 5.3 in order to create 'Summary of findings' tables (RevMan 2014. We produced a summary of the intervention effect and a measure of quality for each of the above outcomes using the GRADE approach. The GRADE approach uses five considerations (study limitations, consistency of effect, indirectness, imprecision, and publication bias) to assess the quality of the body of evidence for each outcome. The quality of the evidence can be downgraded from 'high quality' by one level for serious (or by two levels for very serious) limitations, depending on assessments for risk of bias, serious inconsistency, indirectness of evidence, imprecision of effect estimates, or potential publication bias.

Measures of treatment effect

Dichotomous data

For dichotomous data, we presented results as summary risk ratios with 95% confidence intervals.

Continuous data

We used the mean difference if outcomes were measured in the same way between trials. In future updates, if eligible, we will use the standardised mean difference to combine trials that measure the same outcome, but use different methods.

Unit of analysis issues

Our plan was to consider cluster-randomised trials along with individually randomised trials. However, no cluster-randomised controlled trials were identified. Cross-over designs would not be valid in the context of the interventions tested here.

Cluster-randomised trials

In future updates, if appropriate, we will include cluster-randomised trials in the analyses along with individually randomised trials. We will adjust either their sample sizes or standard errors using the methods described in the *Cochrane Handbook of Systematic Reviews of Interventions,* Section 16.3.4 or 16.3.6, using an estimate of the intracluster correlation co-efficient (ICC) derived from the trial (if possible), from a similar trial, or from a study of a similar population. If we use ICCs from other sources, we will report this, and conduct sensitivity analyses to investigate the effect of variation in the ICC. If we identify both cluster-randomised trials and individually-randomised trials, we plan to synthesise the relevant information. We will consider it reasonable to combine the results from both if there is little heterogeneity between the study designs, and the interaction between the effect of the intervention and the choice of randomisation unit is considered to be unlikely.

We will also acknowledge heterogeneity in the randomisation unit, and perform a sensitivity analysis to investigate the effects of the randomisation unit.

Dealing with missing data

We noted levels of attrition for included studies.

We conducted analyses for all outcomes, as far as possible, on an intention-to-treat basis, i.e. we attempted to include all participants randomised to each group in the analyses. The denominator for each outcome in each trial was the number randomised, minus any participants whose outcomes were known to be missing.

Assessment of heterogeneity

We assessed statistical heterogeneity in each meta-analysis using the Tau^2, I^2, and Chi^2 statistics. We regarded heterogeneity as substantial if I^2 was greater than 30%, and either Tau^2 was greater than zero, or $P < 0.10$ in the Chi^2 test for heterogeneity. If we identified substantial heterogeneity (above 30%), we planned to explore it by pre-specified subgroup analysis.

Assessment of reporting biases

In future updates, if there are 10 or more studies in the meta-analysis, we will investigate reporting biases (such as publication bias) using funnel plots. We will assess funnel plot asymmetry visually. If asymmetry is suggested by a visual assessment, we will perform exploratory analyses to investigate it.

Data synthesis

We carried out statistical analysis using the Review Manager 5 software (RevMan 2014). Given the likely differences in the studied populations and different skill levels of the operators, we decided to apply random-effects for all analyses. In other words, we could not assume that studies were estimating the same underlying treatment effect, i.e. that trials were examining the same intervention, and the trials' populations and methods were sufficiently similar.

We presented the results as the average treatment effect with 95% confidence intervals, and the estimates of Tau^2 and I^2.

Subgroup analysis and investigation of heterogeneity

Given the small number of studies included, we did not perform any subgroup analyses. We felt that any such analyses would be too underpowered to be clinically meaningful.

Sensitivity analysis

Given the small number of studies, we did not perform any sensitivity analyses. We felt that any such analyses would be too underpowered to be clinically meaningful.

RESULTS

Description of studies

Results of the search

See Figure 1.

For this update, we assessed four new reports. We excluded one trial, and the remaining three were reports related to studies included in the original review. Therefore, for this update we still had 16 included studies, and 19 excluded studies.

Included studies

We included a total of 16 randomised studies (Ammala 1993 (MRC Finland); Borrell 1999; Bovicelli 1986; Brambati 1991; Canada 1989; CEMAT 1998; Leiden 1998; MRC 1991; Nicolaides 1994 (King's); Nolan 1981; Philip 2004 (NICHD EATA); Smidt-Jensen 1993 (Denmark); Sundberg 1997 (Copenhagen); Tabor 1986; Tomassini 1988; Jackson 1992).

(1) Second trimester amniocentesis versus control (no testing)

Tabor 1986 was a multi-centre study that included low-risk Danish women aged 25 to 34 years, between 1980 and 1984. Seventy-three per cent (4606/6305) of all eligible women took part. Five doctors performed all procedures; the most experienced operator performed 54%. Amniocentesis was performed with a full bladder, using a linear 3.5 MHz transducer with a channel guide for the needle in the middle of the probe. A 20-gauge needle (0.9 mm outer diameter) was passed through the channel, creating an angle of 90° between the needle and the linear probe.

Tabor 1986 did not report conflicts of interest. This study was supported by the Dagmar Marshall Foundation and was conducted in hospitals in Denmark.

(2) Early versus second trimester amniocentesis

CEMAT 1998 was a multi-centre trial, carried out under the auspices of the Medical Research Council of Canada, with enrolment from July 1994 to December 1996 and follow-up finishing in 1997. It involved 12 participating centres in Canada. Both early and mid-trimester amniocenteses were done with a freehand technique, using a 22-gauge needle under continuous ultrasound guidance. Each operator had done at least 30 early amniocenteses before participating. Eleven millilitres of amniotic fluid were aspirated during early amniocentesis, and 20 mL during second trimester amniocentesis. No more than two attempts were carried out on the same day.

No conflict of interest were reported for the primary researchers.

(3) Chorionic villus sampling (CVS) versus amniocentesis

Four trials compared transcervical CVS with second trimester amniocentesis (Borrell 1999; Canada 1989; MRC 1991; Smidt-Jensen 1993 (Denmark)). The settings for the studies were hospitals in Barcelona (Borrell 1999), multiple centres in Canada (Canada 1989), 31 centres in Europe (MRC 1991), and two hospitals in Denmark (Smidt-Jensen 1993 (Denmark)).

In the Canada 1989 trial, women allocated to have CVS had the transcervical procedure, while in the MRC 1991 trial, CVS was carried out in whichever procedure was deemed suitable by the obstetrician (72% by the transcervical, and 28% by the transabdominal approach). In the MRC 1991 trial, of the 1592 women randomised to amniocentesis with follow-up data, 1417 (89%) were known to have had an amniocentesis. In the Finnish arm of the MRC trial, all CVS procedures were carried out by the transcervical approach. In the Canada 1989 trial, a pre-entry ultrasound could not be performed in all centres. As a consequence, 14.2% of women with non-viable, multiple, or advanced pregnancies were subsequently excluded, after randomisation, from some analyses. The Smidt-Jensen 1993 (Denmark) trial was designed as a three-way randomisation of women classified as low genetic risk (transabdominal CVS versus transcervical CVS versus amniocentesis). Borrell 1999 randomised women to transcervical CVS (9 to 13 weeks), or amniocentesis (15 to 18 weeks). This trial was stopped prematurely when second trimester biochemistry screening was introduced.

No trial reported if authors had conflicts of interest. Borrell 1999 did not report the dates of the study. Canada 1989 reported study dates to be October 1987 to September 1988; MRC 1991 recruited from 1985 to 1989; and Smidt-Jensen 1993 (Denmark) study dates were August 1985 to October 1990. Sources of funding were not reported in Borrell 1999; Canada 1989 was supported by the Canadian Medical Research Council; MRC 1991 was supported by a grant from the Department of Health, UK; and Smidt-Jensen 1993 (Denmark) was supported by grants from Gangstedfonden, Egmont H. Petersens Fond, Fru Lily Benthine Lunds Fond, Rosalie Petersens Fond, Meda A/S, Bruel og Kjaer, S&W Fondet, Tuborgfondet, Unisis Corp., Winterthur-borgen Legatet, Hafuia Fonden, Kromosomforskningsfonden, and the US National Institute of Child Health and Human Development.

(4) Chorionic villus sampling (CVS) trials

Jackson 1992 was a large multi-centre collaborative study, carried out in a hospital setting in the US, conducted from April 1987 to September 1989, under the auspices of the US National Institute of Child Health, comparing transabdominal and transcervical CVS. In total, 3999 women were randomised. Transcervical CVS was performed with a 1.5 mm plastic catheter, and abdominal procedure with a spinal needle (18- to 22-gauge). Brambati 1991, carried out in a hospital in Milan, randomised 78.6% of eligible women referred for genetic counselling at six to eight weeks' gestation. A single operator performed all procedures (both transabdominal and transcervical). Transcervical CVS was performed using a cannula with an outer diameter of 1.45 mm, and the transabdominal procedure was done with a spinal needle (1.1 mm outer diameter). A maximum of two passes was allowed in one sampling session. Bovicelli 1986 reported the results of his study in a letter to the *Lancet*. His study was carried out in a hospital in Bologna. Transcervical CVS was performed using a flexible 16-gauge silver cannula. The transabdominal procedure was carried out with a double-needle system, with an 18-gauge guide needle, and a 21-gauge aspiration needle. Tomassini 1988 was a single-centre trial from a hospital setting in Varese (Italy), in which 44 women were assigned to transcervical or transabdominal procedure by 'random selection'. Smidt-Jensen 1993 (Denmark), carried out in two hospitals in Denmark, randomised women at high genetic risk to either transabdominal or transcervical CVS.

No trial reported if authors had conflicts of interest. Brambati 1991 was conducted from March 1986 to July 1988 and was partially supported by the WHO Hereditary Disease Programme. Study

dates and funding sources were not reported in Bovicelli 1986 or Tomassini 1988. Jackson 1992 was conducted between April 1987 and September 1989, but did not report any sources of funding.

(5) Early amniocentesis versus transabdominal CVS

Four completed randomised controlled trials have been identified so far.

The NICHD EATA Group Trial was a large multi-centre collaborative study carried out between 1997 and 2000 in hospitals in Denmark, US, and Canada, under the auspices of the US National Institute of Child Health and Human Development, and the Centre for Evaluation and Health Technology Assessment of the Danish National Board of Health (Philip 2004 (NICHD EATA)). The trial randomised 3775 women, of the 3803 eligible women who consented to participate, from a total group of 6370 women who were screened for eligibility. Eighty-seven per cent of the women were randomised at Rigshospitalet, Denmark, 7% at 11 U.S. centres, and 6% at two Canadian centres. In the early amniocentesis group, a 22-gauge spinal needle was used, and 1 mL of amniotic fluid was aspirated for each week of pregnancy. In the CVS group, a single- (19- to 20-gauge) or double-needle technique (18- to 20-gauge) was used, with the larger 'guide' needle introduced to the margin of the chorion, followed by the sample needle passing through the guide needle into the villi. To participate in the trial, operators were required to have completed at least 25 amniocenteses and 25 transabdominal CVS, on women between 77 and 104 days of gestation. Thirty-two operators were certified to perform procedures at the 14 clinical centres. Two sampling passes were allowed. If required, a second procedure could only be performed seven days after the first attempt.

In the Nicolaides 1994 (King's) and Leiden 1998 trials, recruited women were given the choice between early amniocentesis, transabdominal CVS, or randomisation. In the Nicolaides 1994 (King's) trial, carried out in a hospital in London, 37% opted for randomisation (555/1492), 38% for early amniocentesis (562/1492), and 25% for CVS (375/1492). In the Leiden 1998 trial, carried out in Leiden University Hospital, the Netherlands, 55% of women were randomised (115/210), 33% chose early amniocentesis, and 12% chose CVS.

The procedure for transabdominal CVS was similar in three included trials. Nicolaides 1994 (King's) and Leiden 1998 used a 20-gauge needle. The tip of the needle was moved five to 10 times while applying negative pressure by manual aspiration through a 20 mL syringe. In the Sundberg 1997 (Copenhagen) trial, a double-needle technique was used, with a guide needle of 1.2 mm (18-gauge) and an aspiration needle of 0.8 mm (21-gauge).

There were important differences in the early amniocentesis technique used in Sundberg 1997 (Copenhagen), compared to Nicolaides 1994 (King's) and Leiden 1998. In Sundberg 1997 (Copenhagen), carried out in a University Hospital, the filter system was used, which allowed re-injection of the majority of the entire aspirated volume, back into the amniotic cavity. Early amniocentesis in the Nicolaides 1994 (King's) and Leiden 1998 trials was done by straightforward aspiration of 11 mL of amniotic fluid, the first 1 mL of which was discarded. Nicolaides 1994 (King's) used a 20-gauge, and Leiden 1998 used a 22-gauge needle.

None of the studies reported conflicts of interest for any of the primary researchers and three studies did not declare sources of funding in the trial reports(Leiden 1998; (Nicolaides 1994 (King's); Sundberg 1997 (Copenhagen). Dates of the study for Nicolaides 1994 (King's) were from January 1990 to March 1993 London; dates were not specified in Leiden 1998; and in Sundberg 1997 (Copenhagen), study dates were from February 1993 to September 1995.

(6) Use of ultrasounds

Nolan 1981 compared ultrasound-directed taps with taps inserted without the benefit of ultrasound scans. Amniocenteses in the experimental' group were not ultrasound-guided in the true meaning of this term. Today, the term 'ultrasound-guided procedure' is used to describe needle insertion under simultaneous ultrasound guidance, using either a freehand technique, or a needle guide mounted on the ultrasound probe. In the study by Nolan 1981, scans were performed before the procedure, with the main aim to inform the operator of the placental position. The physician who had the benefit of the ultrasound report, attempted to avoid the placenta. In the control group, the physician selected what he or she considered the best site for introduction of the needle.

Nolan 1981 was conducted in a hospital setting in the US. Funding sources, study dates and conflicts of interest of the primary researchers were not reported.

Excluded studies

We excluded 19 studies from the review. Eleven studies did not evaluate the intervention or comparison criteria specified in this review (Corrado 2002; Fischer 2000; Gordon 2007; Hewison 2006 (ARIA Trial); Leach 1978; Leung 2002; Pistorius 1998; SIlver 2005; Van Schoubroeck 2000; Wax 2005), eight studies were not randomised trials (Cederholm 1997 (Uppsala); Chang 1994; Horovitz 1994; Ketupanya 1997; Levine 1977; Shalev 1994; Shulman 1990; Zwinger 1994).

We identified a new trial by ISRCTN18010960 in the searches for this update. We excluded it, as the trial assessed the use of amniocentesis for identification of rapid markers of subclinical chorioamnionitis prior to cervical cerclage versus cervical cerclage alone, in a population of women deemed to have cervical incompetence. This population was outside the scope of this review.

Risk of bias in included studies

Please see Figure 2; Figure 3 for summaries of 'Risk of bias' assessments.

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias domain presented as percentages across all included studies.

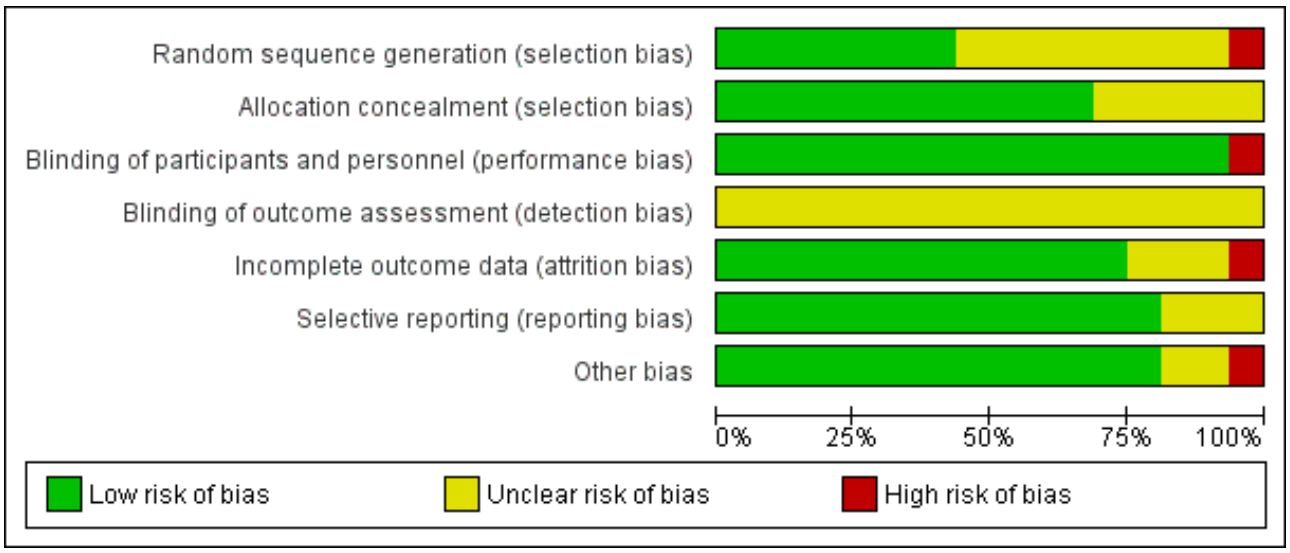
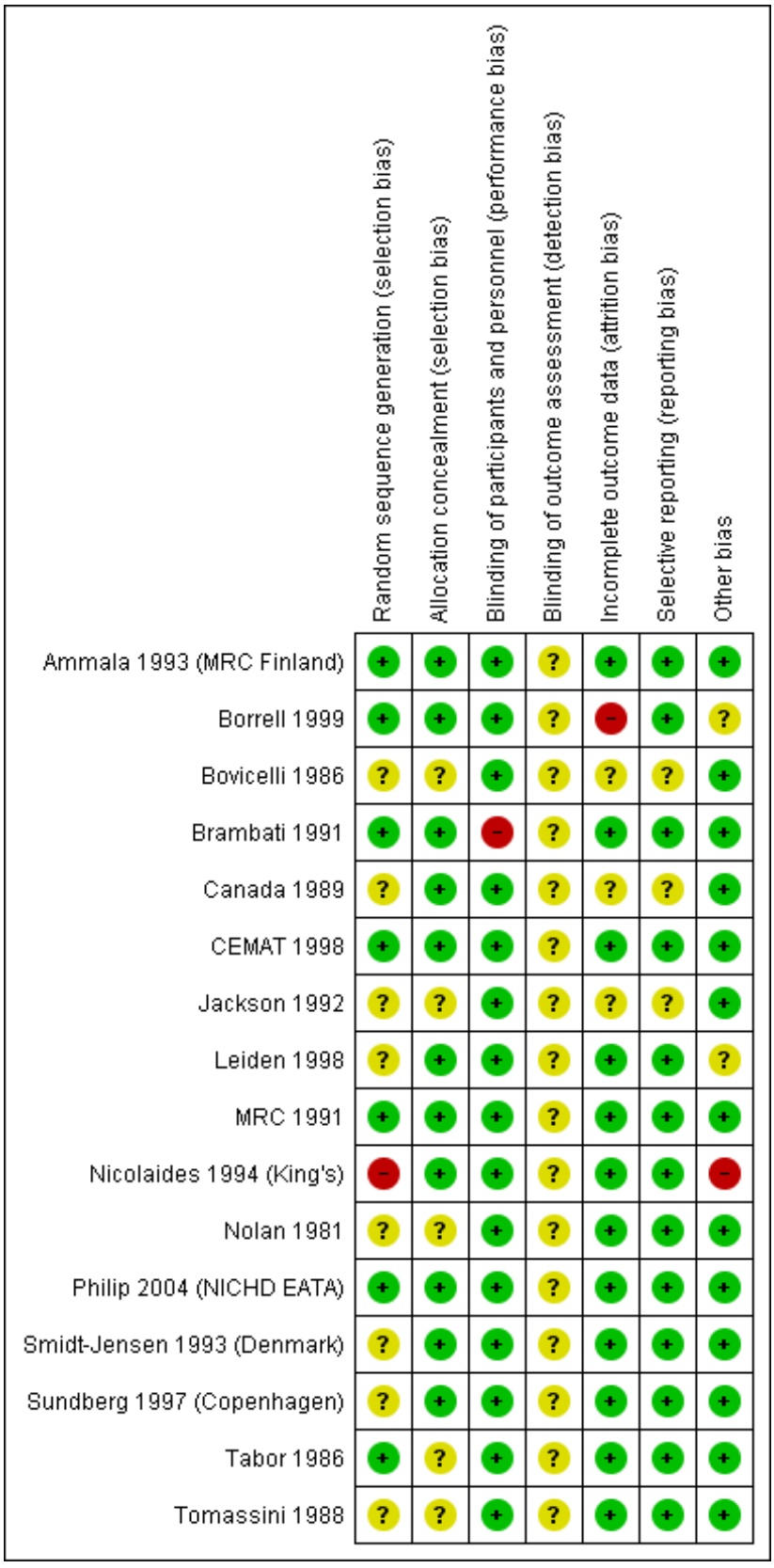


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias domain for each included study.



Allocation

We judged seven studies to be at low risk of bias for random sequence generation (Ammala 1993 (MRC Finland); Borrell 1999; Brambati 1991; CEMAT 1998; MRC 1991; Philip 2004 (NICHD EATA); Tabor 1986) – all described recognised methods for random sequence generation. We judged eight studies to be at unclear risk of bias for random sequence generation, as they did not specify the method used (Bovicelli 1986; Canada 1989; Leiden 1998; Nolan 1981; Smidt-Jensen 1993 (Denmark); Sundberg 1997 (Copenhagen); Tomassini 1988; Jackson 1992). One study described randomisation by offering a choice of two non-sequentially numbered envelopes to participants, which was not a recognised method for random sequence generation (Nicolaides 1994 (King's)).

We judged 11 studies to be at low risk of bias for allocation concealment; studies either stored allocation information centrally, used telephone allocation, or concealed allocation in sequentially numbered envelopes (Ammala 1993 (MRC Finland); Borrell 1999; Brambati 1991; Canada 1989; CEMAT 1998; Leiden 1998; MRC 1991; Nicolaides 1994 (King's); Philip 2004 (NICHD EATA); Smidt-Jensen 1993 (Denmark); Sundberg 1997 (Copenhagen)). We judged five studies to be at unclear risk, as they did not specify methods used for allocation concealment (Bovicelli 1986; Nolan 1981; Tabor 1986; Tomassini 1988; Jackson 1992).

Blinding

Due to the nature of the comparisons assessed, we judged that it was not feasible to blind participants and personnel, and we deemed 15 studies to be at low risk of performance bias (Ammala 1993 (MRC Finland); Borrell 1999; Bovicelli 1986; Canada 1989; CEMAT 1998; Leiden 1998; MRC 1991; Nicolaides 1994 (King's); Nolan 1981; Philip 2004 (NICHD EATA); Smidt-Jensen 1993 (Denmark); Sundberg 1997 (Copenhagen); Tabor 1986; Tomassini 1988; Jackson 1992). We considered one study to be at high risk, as the proportion of cases in which the operator deviated from the allocated procedure increased during the study (4.6% in year one, 9.7% in year two, 15.5% in year three). The majority of deviations were in the transcervical CVS arm, where deviations were 6% in year one, 16% in year two, and 27% in year three (Brambati 1991). We judged all included studies to be at unclear risk of detection bias, as none reported blinding of outcome assessors.

Incomplete outcome data

We judged 12 studies to be at low risk of attrition bias, as they appropriately reported outcome data (Ammala 1993 (MRC Finland); Brambati 1991; CEMAT 1998; Leiden 1998; MRC 1991; Nicolaides 1994 (King's); Nolan 1981; Philip 2004 (NICHD EATA); Smidt-Jensen 1993 (Denmark); Sundberg 1997 (Copenhagen); Tabor 1986; Tomassini 1988). We judged three studies to be at unclear risk. For Bovicelli 1986, outcome data were reported briefly, in letter format only. For Canada 1989, outcomes were not reported for 72 (5.3%) participants randomised to CVS and 90 (6.6%) randomised to amniocentesis. There were 22 participants lost to follow-up in the CVS and and 30 participants lost in the amniocentesis groups. In Jackson 1992, for the majority of important clinical outcomes including type of pregnancy loss, intention-to-treat analysis was not feasible because data were presented only for women with genetically normal pregnancies (91.5%). One study was at high risk, as the assigned procedure was performed in 681/1011 women (Borrell 1999). A large and uneven dropout rate may be a source of significant bias, and data from this trial have to be interpreted with caution.

Selective reporting

We assessed thirteen studies to be at low risk of selective reporting, as outcomes were appropriately addressed (Ammala 1993 (MRC Finland); Borrell 1999; Brambati 1991; CEMAT 1998; Leiden 1998; MRC 1991; Nicolaides 1994 (King's); Nolan 1981; Philip 2004 (NICHD EATA); Smidt-Jensen 1993 (Denmark); Sundberg 1997 (Copenhagen); Tabor 1986; Tomassini 1988). We judged three studies to be at unclear risk: Bovicelli 1986 reported outcomes in letter format, Canada 1989 did not report outcomes for 72 (5.3%) participants randomised to CVS and 90 (6.6%) randomised to amniocentesis, and Jackson 1992 did not report outcomes for women for whom sampling was not attempted (3.2%).

Other potential sources of bias

Thirteen studies showed no evidence of being impacted by other sources of bias (Ammala 1993 (MRC Finland); Bovicelli 1986; Brambati 1991; Canada 1989; CEMAT 1998; MRC 1991; Nolan 1981; Philip 2004 (NICHD EATA); Smidt-Jensen 1993 (Denmark); Sundberg 1997 (Copenhagen); Tabor 1986; Tomassini 1988; Jackson 1992). We found two studies to be at unclear risk of other bias. Borrell 1999 was prematurely discontinued when second trimester serum biochemistry screening was introduced. We were unsure of the impact of insufficient recruitment to fulfil sample size calculation. In Leiden 1998, the number of women who did not receive the intervention according to allocation was not evenly distributed between the groups, and may indicate impact of bias. We judged Nicolaides 1994 (King's) to be at high risk of other bias, as the trial had to be stopped due to adverse publicity regarding risks associated with CVS.

Effects of interventions

See: **Summary of findings for the main comparison** Second trimester amniocentesis compared to control for prenatal diagnosis; **Summary of findings 2** Early compared to second trimester amniocentesis for prenatal diagnosis; **Summary of findings 3** Transabdominal chorionic villus sampling compared to second trimester amniocentesis for prenatal diagnosis; **Summary of findings 4** Transcervical compared to transabdominal chorionic villus sampling for prenatal diagnosis; **Summary of findings 5** Early amniocentesis compared to transabdominal chorionic villus sampling for prenatal diagnosis

Please note, all analyses were carried out using a random-effects model and so the average treatment effect is presented for all results.

(1) Second trimester amniocentesis versus control (no testing)

Only one study that included 4606 women provided data for this comparison Tabor 1986).

Primary outcomes

Tabor 1986 provided the best estimate of an excess pregnancy loss in low-risk women caused by amniocentesis (3.2% versus 2.3%; RR 1.41, 95% CI 0.99 to 2.00; Analysis 1.1; moderate-quality evidence), and spontaneous miscarriage (2.1% versus 1.3%; RR 1.60, 95% CI 1.02 to 2.52; Analysis 1.2; high-quality evidence). However, the confidence intervals around the estimate for pregnancy loss were

relatively wide, and were compatible with no or minimal excess risk. It is important to note that 95% CI for absolute risk difference ranged from 0% to 2% for both outcomes. Spontaneous miscarriage after test (pregnancy loss in women who had the test actually performed) was not reported.

Secondary outcomes

There was no clear difference in vaginal bleeding between the two groups (2.4% versus 2.5%; RR 0.95, 95% CI 0.66 to 1.37; Analysis 1.8), but amniotic fluid leakage was more common after amniocentesis (1.7% versus 0.4%; RR 3.90; 95% CI 1.95 to 7.80; Analysis 1.9).

There was more non-compliance in the amniocentesis group (1.7% versus 1.0%; RR 1.73; 95% CI 1.03 to 2.91; Analysis 1.3). In order to quantify complications related to the procedure, we reported multiple insertions (2.0% versus 0.0%; RR 91.08, 95% CI 5.61 to 1477.53; Analysis 1.4), need for the second test (0.9% versus 0.0%; RR 41.04, 95% CI 2.48 to 678.07; Analysis 1.5), laboratory failure (0.6% versus 0.0%; RR 27.02, 95% CI 1.61 to 454.31; Analysis 1.6; high-quality evidence), and all non-mosaic abnormalities (0.7 versus 0.0; RR 30.85, 95% CI 1.85 to 515.31; Analysis 1.7).

There were potentially clinically important differences in termination of all pregnancy (0.7% versus 0.3 %; RR 2.5, 95% CI 0.97 to 6.44; Analysis 1.10); and perinatal deaths (0.4% versus 0.7%; RR 0.63, 95% CI 0.28 to 1.38; Analysis 1.11), stillbirths (0.4% versus 0.5%; RR 0.83, 95% CI 0.36 to 1.93; Analysis 1.12), neonatal deaths (0.0% versus 0.2%; RR 0.83, 95% CI 0.36 to 1.93; Analysis 1.13), all recorded deaths after viability (0.4% versus 0.7%; RR 0.63, 95% CI 0.28 to 1.38; Analysis 1.14), anomalies (2.0% versus 2.2%; RR 0.93, 95% CI 0.62 to 1.39; Analysis 1.15; moderate-quality evidence), talipes (0.8% versus 1.2%; RR 0.68, 95% CI 0.37 to 1.22; Analysis 1.16), neonatal respiratory distress syndrome (1.1% versus 0.5%; RR 2.11, 95% CI 1.06 to 4.19, Analysis 1.17), but the number of events were not large enough to precisely estimate the risks.

The following secondary outcomes were not reported.

- Sampling failure.
- All mosaics.
- True mosaics.
- Confined mosaics.
- Maternal contamination.
- Known false positive after birth.
- Known false negative after birth.
- Reporting time (interval between sampling and result).
- Vaginal bleeding after 20 weeks.
- pre-labour ruptured membranes before 28 weeks.
- Antenatal hospital admission.
- Delivery before 37 weeks.
- Delivery before 33 weeks.
- Talipes equinovarus (the foot is plantar flexed, inverted, and markedly adducted).
- Haemangiomas (localised vascular lesions of the skin and subcutaneous tissue).
- Limb reduction defects.
- Admission to special care baby unit.
- Birthweight below the 10th centile.
- Birthweight below the 5th centile.

(2) Early versus second trimester amniocentesis

One trial with 4334 women contributed to this comparison (CEMAT 1998)

Primary outcomes

Compared to an early amniocentesis, mid-trimester procedure was safer; total pregnancy loss after early amniocentesis was higher (7.6% versus 5.9%; RR 1.29, 95% CI 1.03 to 1.61; Analysis 2.1; high-quality evidence). There were also more spontaneous miscarriages in the early amniocentesis group (3.6% versus 2.5%; RR 1.41, 95% CI 1.00 to 1.98; Analysis 2.2; moderate-quality evidence). This was also true for spontaneous miscarriages after test (2.5% versus 0.8%; RR 3.22, 95% CI 1.88 to 5.53; Analysis 2.3).

Secondary outcomes

The number of congenital anomalies was higher in the early amniocentesis group (4.7% versus 2.7%; RR 1.73, 95% CI 1.26 to 2.38, Analysis 2.19; high-quality evidence). If one restricted the analysis to women who actually had early amniocentesis ('on treatment' analysis), the risk of talipes was even higher (1.3% versus 0.1%; RR 14.43, 95% CI 3.45 to 60.41; Analysis 2.20).

Early amniocentesis required more multiple needle insertions compared with mid-trimester amniocentesis (4.7% versus 1.7%; RR 2.79, 95% CI 1.92 to 4.04; Analysis 2.6). Early amniocentesis was also more demanding for cytogeneticists with 1.8% laboratory failures after early procedure and only 0.2% after mid-trimester amniocentesis (RR 9.76, 95% CI 3.49 to 27.26; Analysis 2.8; high-quality evidence). There were three known false negative after birth cytogenetic results in the early amniocentesis group and none after mid-trimester amniocentesis (0.05% versus 0.0%; RR 3.00, 95% CI 0.12 to 73.67; Analysis 2.12; moderate-quality evidence). Two reports resulted in incorrect information about sex chromosomes, and in one case, a very subtle chromosome abnormality at the terminal end of chromosome one was missed and detected postnatally (0.1% versus 0.0%; RR 5.0, 95% CI 0.24 to 104.18; Analysis 2.12; moderate-quality evidence). Interestingly, a known false positive after birth rate was reported to be 3.6% for early amniocentesis and 8% for mid-trimester amniocentesis. We could not extract the actual numbers from the trial reports, so we did not show this outcome in the outcome table. It appeared that most of these known false positive after birth results were so called 'pseudomosaics', not reported to the physicians. There were more cases of amniotic leakage after test in the early trimester amniocentesis group (4.0% versus 2.0%; RR 2.05, 95% CI 1.43 to 2.94; Analysis 2.14). Those who did not comply with allocated procedures were more numerous in the second trimester amniocentesis group (12.2% versus 18.8%; RR 0.65, 95% CI 0.57 to 0.75; Analysis 2.4).

There were some potentially important differences in sampling failure (1.5% versus 0.3%; RR 4.53, 95% CI 0.53 to 38.56; Analysis 2.5; moderate-quality evidence), spontaneous miscarriage (3.6% versus 2.5%; RR 1.41, 95% CI 1.0 to 1.98; Analysis 2.2; moderate-quality evidence); maternal contamination (0.2% versus 0.1%; RR 2.0, 95% CI 0.4 to 10.92; Analysis 2.11); termination of pregnancy (3.5% versus 2.6%; RR 1.26, 95% CI 0.89 to 1.77; Analysis 2.15), neonatal deaths (0.2% versus 0.04%; RR 4.98, 95% CI 0.58 to 42.56; Analysis 2.17), stillbirths (0.5% versus 0.7%; RR 0.73, 95% CI 0.34 to 1.59; Analysis 2.16), but the number of events were not large enough to be able to precisely estimate the risks.

Other included outcomes were fairly similar, but some 95% CI remain quite imprecise (all non-mosaic abnormalities, Analysis 2.9; reporting time, Analysis 2.13; and all deaths reported after viability, Analysis 2.18).

The following secondary outcomes were not reported.

- All mosaics.
- Confined mosaics.
- Known false positive after birth.
- Vaginal bleeding after test.
- Vaginal bleeding after 20 weeks.
- pre-labour ruptured membranes before 28 weeks.
- Antenatal hospital admission.
- Delivery before 37 weeks.
- Delivery before 33 weeks.
- Perinatal mortality (stillbirths and neonatal deaths in the first week of life).
- Haemangiomas (localised vascular lesions of the skin and subcutaneous tissue).
- Limb reduction defects.
- Admission to special care baby unit.
- Neonatal respiratory distress syndrome.
- Birthweight below the 10th centile.
- Birthweight below the 5th centile.

(3) Chorionic villus sampling (CVS) versus amniocentesis

3.1. Transcervical CVS versus second trimester amniocentesis

Four trials with 6527 women compared transcervical CVS with second trimester amniocentesis (Ammala 1993 (MRC Finland); Borrell 1999; Canada 1989; Smidt-Jensen 1993 (Denmark)).

Primary outcomes

Total pregnancy loss was higher after transcervical CVS compared with amniocentesis (14.5% versus 11.0%; RR 1.40, 95% CI 1.09 to 1.81; women = 6527; studies = four; Analysis 3.1), but these results should be interpreted cautiously. In the transcervical CVS group, the total pregnancy loss varied from 7.3% in the Ammala 1993 (MRC Finland) trial to 19.5% in the Borrell 1999 trial. It is important to note that this was an intention-to-treat analysis, which included post-randomisation, pre-procedure pregnancy losses. Unfortuntaely, in the Borrell 1999 trial, these losses were extremely high (10.9%) and unbalanced between the two groups. The overall results for total pregnancy loss changed little without Borrell 1999 (RR 1.40, 95% CI 1.00 to 2.06). Interestingly, the statistical test for heterogeneity was significant, despite the fact that the results looked quite similar in terms of the size and direction of the observed differences in total pregnancy loss. The sensitivity analysis suggested that the heterogeneity was caused by the differences between the two largest trials (Canada 1989; Smidt-Jensen 1993 (Denmark)). The increase in pregnancy loss after transcervical CVS was clear in the Smidt-Jensen 1993 (Denmark) trial (RR 1.70, 95% CI 1.30 to 2.22), but not in the Canada 1989 trial (1.10, 95% CI 0.92 to 1.30). The results for spontaneous miscarriages (12.9% versus 9.4%; RR 1.5, 95% CI 1.07 to 2.11; studies = three; women = 5506; Analysis 3.2) were consistent with the results for total pregnancy loss described above.

There was no clear difference between groups for spontaneous miscarriage after test (pregnancy loss in women who had the test actually performed (RR 1.77, 95% CI 0.28 to 11.00; studies = two; women = 1579; Analysis 3.3).

Secondary outcomes

For technical difficulties in sampling, the transcervical CVS group had more multiple insertions (30.8% versus 7.8%; RR 3.93, 95% CI 2.72 to 5.68; studies = one; women = 794; Analysis 3.6), and second tests (6.3% versus 0.2%; RR 19.63, 95% CI 1.24 to 309.9; studies = three; women = 4256; Analysis 3.7), while there were no clear differences between groups for non-compliance with allocated procedure (RR 0.51, 95% CI 0.14 to 1.87; studies = three; women = 4595; Analysis 3.4), or sampling failure (RR 0.55, 95% CI 0.26 to 1.19; studies = one; women = 797; Analysis 3.5).

For cytogenetic analysis, the transcervical CVS group had more laboratory failure (1.7% versus 0.1%; RR 22.62, 95% CI 3.07 to 166.89; studies = two; women = 2792; Analysis 3.8), and confined mosaics (2.3% versus 0.4%; RR 5.66, 95% CI 1.97 to 16.24; studies = one; women = 1995; Analysis 3.11), and maternal contamination (3.8% versus 0.3%; RR 12.3, 95% CI 3.81 to 39.67; studies = one; women = 1991; Analysis 3.12).

For pregnancy complications, the transcervical CVS group had more vaginal bleeding after test (19.4% versus 2.4%; RR 11.48, 95% CI 2.58 to 51.08; studies = two; women = 3193; Analysis 3.19), and pre-labour ruptured membranes before 28 weeks (4.1% versus 0.8%; RR 4.97, 95% CI 1.45 to 17.03; studies = one; women = 722; Analysis 3.22).

There were a number of outcomes with potentially clinically important differences, but the number of events were not large enough to precisely estimate the risks. These were: termination of pregnancy (all; 2.4% versus 2.7%; RR 0.88, 95% CI 0.58 to 1.34; studies = two; women = 3454, Analysis 3.26), perinatal mortality (stillbirths and neonatal deaths in the first week of life; 0.5% versus 0.3%; RR 1.79, 95% CI 0.42 to 7.69; studies = three; women = 5521; Analysis 3.27), stillbirths (0.3% versus 0.2%; RR 0.94, 95% CI 0.02 to 45.31; studies = two; women = 3454; Analysis 3.28), neonatal death (death in the first week of life; 0.2% versus 0.1%; RR 1.63, 95% CI 0.38 to 7.05; studies = three; women = 4251; Analysis 3.29), and all recorded deaths after viability (0.5% versus 0.6%; RR 0.78, 95% CI 0.02 to 25.93; studies = two; women = 1579; Analysis 3.30.

The same was true for neonatal complications, such as anomalies (all recorded; 1.0% versus 1.6%; RR 0.62, 95% CI 0.25 to 1.59; studies = two; neonates = 1408; Analysis 3.31), and talipes (clubfoot; RR 0.62, actual numbers not provided; studies = one; neonates = 797).

The following secondary outcomes were not reported.

- All mosaics.
- Reporting time (interval between sampling and result).
- Amniotic leakage after test.
- Delivery before 33 weeks.
- Haemangiomas (localised vascular lesions of the skin and subcutaneous tissue).
- Limb reduction defects.
- Admission to special care baby unit.
- Neonatal respiratory distress syndrome.

- Birthweight below the 10th centile.
- Birthweight below the 5th centile.

3.2. Transabdominal CVS versus second trimester amniocentesis

One trial with 2234 women contributed to this comparison (Smidt-Jensen 1993 (Denmark)).

Primary outcomes

A subgroup of Smidt-Jensen 1993 (Denmark) compared transabdominal CVS with second trimester amniocentesis and found no clear difference in the total pregnancy loss between the two procedures (6.3% versus 7%; RR 0.90, 95% CI 0.66 to 1.23; studies = one; women = 2234; Analysis 3.1; low-quality evidence). The same was true for spontaneous miscarriage (3.0% versus 3.9%; RR 0.77, 95% CI 0.49 to 1.21; studies = one; women = 2069; Analysis 3.2; low-quality evidence).

Spontaneous miscarriage after test (pregnancy loss in women who had the test actually performed) was not reported.

Secondary outcomes

There were no clinically important differences in perinatal mortality (stillbirths and neonatal deaths in the first week of life; 0.7% versus 0.6%; RR 1.18, 95% CI 0.40 to 3.51; studies = one; women = 2069; Analysis 3.27; low-quality evidence), but the number of events were not large enough to precisely estimate the risks. There were no clear differences between groups for amniotic leakage after test (1.4% versus 0.6%; RR 2.53, 95% CI 0.81 to 7.92; studies = one; women = 1485; Analysis 3.20).

The following secondary outcomes were not reported.

- Non-compliance with allocated procedure.
- Sampling failure.
- Multiple insertions.
- Second test performed.
- Laboratory failure.
- All non-mosaic abnormalities.
- All mosaics.
- True mosaics.
- Confined mosaics.
- Maternal contamination.
- Known false positive after birth.
- Known false negative after birth.
- Reporting time (interval between sampling and result).
- Vaginal bleeding after test.
- Vaginal bleeding after 20 weeks.
- pre-labour ruptured membranes before 28 weeks.
- Antenatal hospital admission.
- Delivery before 37 weeks.
- Delivery before 33 weeks.
- Termination of pregnancy (all).
- Stillbirths.
- Neonatal death (death in the first week of life).
- All recorded deaths after viability.
- Anomalies (all recorded).
- Talipes (clubfoot).
- Haemangiomas (localised vascular lesions of the skin and subcutaneous tissue).
- Limb reduction defects.
- Admission to special care baby unit.
- Neonatal respiratory distress syndrome.
- Birthweight below the 10th centile.
- Birthweight below the 5th centile.

3.3. CVS by any route versus second trimester amniocentesis

Primary outcomes

Two trials with 6503 women presented data for the comparison between CVS performed by any route and mid-trimester amniocentesis (MRC 1991; Smidt-Jensen 1993 (Denmark)).

Overall pregnancy loss (including termination of pregnancy) was higher after CVS (11.1% versus 8.2%; RR 1.43, 95% CI 1.22 to 1.67; studies = two; women = 6503; Analysis 3.1). Again, an increase in spontaneous miscarriages after CVS was the main contributing factor (RR 3.46, 95% CI 2.21 to 5.42; studies = one; women = 3201; Analysis 3.3. There were more spontaneous miscarriages in the CVS group (7.1% versus 5.0%; RR 1.51, 95% CI 1.23 to 1.85; studies = two; women = 6280; Analysis 3.2).

Secondary outcomes

Overall, the test had to be repeated more commonly after transcervical CVS compared with second trimester amniocentesis. Also, there were more problems in analysing placental tissue obtained from CVS compared with amniotic fluid. In the transcervical CVS group, laboratory failure occurred in 1.7% cases, compared with only 0.07% after amniocentesis; there were also more cytogenetic abnormalities confined to the placenta, and more known false positive after birth and known false negative after birth results. However, cytogenetic results presented here should be interpreted with caution. They probably underestimate the true incidence of inaccurate results in both the CVS and amniocentesis groups, because the majority of fetal losses were not karyotyped post-mortem, either because of technical difficulties or concerns about medico-legal implications. The lack of complete cytogenetic follow-up in all trials made unbiased analyses on all randomised women impossible.

Complications were uncommon after both procedures, and there were no reports that these were ever life-threatening. Vaginal bleeding following the procedure was more common after transcervical CVS, although there was no difference in the incidence of vaginal bleeding later in pregnancy. There was no significant difference in the amniotic fluid leakage following the procedure, and pre-labour spontaneous rupture of membranes before 28 weeks in MRC 1991, but this observation should be interpreted cautiously, because data on ruptured membranes were missing for large numbers of women. Interestingly, one participating centre reported a significant increase in ruptured membranes after transcervical CVS (Ammala 1993 (MRC Finland)). No differential effect was detected on antenatal admission to hospital.

In the sub-project of the Canada 1989 trial, Spencer 1987, Spencer 1988, and Robinson 1988 compared the psychological effects of transcervical CVS and amniocentesis. In mid-pregnancy, women allocated to amniocentesis were more anxious, and felt less attachment to their babies, although by 22 weeks, these differences

seemed to disappear (data were not available in a form suitable to include in a meta-analysis). Nevertheless, at 22 weeks, there was a suggestion of a persistent differential effect manifested in a decreased desire for another child, associated with amniocentesis (7/26 in the CVS group compared with 13/25 after amniocentesis).

Possible links between CVS, amniocentesis, and congenital anomalies could not be fully explored because of incomplete reporting, and a relatively small number of participants. There have been several reports in the past suggesting the presence of congenital anomalies (limb deformities in particular) in infants exposed to CVS in the first trimester. The available data from included studies did not support this observation. However, it should be remembered that the relationship may be gestation-dependent. The majority of procedures were carried out after nine weeks' gestation, and therefore, did not address the possibility that CVS carried out very early in pregnancy may increase the risk of congenital abnormalities.

For technical difficulties in sampling, the CVS group had more sampling failure (4.8% versus 1.6%; RR 3.09, 95% CI 1.98 to 4.82; studies = one; women = 3201; Analysis 3.5), multiple insertions (30.7% versus 6.3%; RR 4.85, 95% CI 3.92 to 6.01; studies = one; women = 2917; Analysis 3.6), and second tests performed (6.2% versus 2.1%; RR 2.83, 95% CI 1.94 to 4.13; studies = one; women = 3201; Analysis 3.7). There were no clear differences between the groups for numbers of laboratory failures (RR 0.77, 95% CI 0.29 to 2.06; studies = one; women = 3201; Analysis 3.8), or known false positive after birth results (RR 0.99, 95% CI 0.06 to 15.80; studies = one; women = 3201; Analysis 3.13).

Reporting time for cytogenetic analyses was analysed in weekly tranches, although we did not pre-specify these particular time intervals. The CVS group had more results given in less than seven days (15.2% versus 0.6%; RR 23.52, 95% CI 12.54 to 44.10; studies = one; women = 3099; Analysis 3.15), less than 14 days (22.5% versus 5.7%; RR 3.96, 95% CI 3.17 to 4.95; studies = one; women = 3099; Analysis 3.16), and fewer results given at more than 21 days (10.8% versus 32.6%; RR 0.33, 95% CI 0.28 to 0.39; studies = one; women = 3099; Analysis 3.18).

For pregnancy complications, the CVS group had more deliveries at less than 37 weeks (18.3% versus 13.7%; RR 1.33, 95% CI 1.13 to 1.57; studies = one; women = 3189; Analysis 3.24).

There were a number of outcomes with potentially clinically important differences, but the number of events was not large enough to precisely estimate the risks. These were: termination of pregnancy (all; 3.7% versus 2.6%; RR 1.42, 95% CI 0.96 to 2.11; studies = one; women = 3201; Analysis 3.26), perinatal mortality (stillbirths and neonatal deaths in the first week of life; 0.7% versus 0.6%; RR 1.20, 95% CI 0.64 to 2.24; studies = two; neonates = 6280; Analysis 3.27), stillbirths (0.4% versus 0.4%; RR 0.99, 95% CI 0.35 to 2.81; studies = one; neonates = 3201; Analysis 3.28), neonatal death (death in the first week of life; 0.5% versus 0.2%; RR 2.64, 95% CI 0.7 to 9.93; number of studies = one; neonates = 3201; Analysis 3.29), all recorded deaths after viability (1.0% versus 0.7%; RR 1.44; 95% CI 0.67 to 3.09; studies = one; neonates = 3201; Analysis 3.30), anomalies (all recorded; 5.9% versus 7.2%; RR 0.77, 95% CI 0.66 to 0.89; studies = two; number of neonates = 3338; Analysis 3.31), haemangiomas (localised vascular lesions of the skin and subcutaneous tissue; 29.4% versus 21.8%; RR 1.35, 95% CI 0.81 to 2.24; studies = one; neonates = 182; Analysis 3.32), and limb reduction defects (0.1% versus 0.0%; RR 4.95, 95% CI 0.24 to 102.97; studies = one; neonates = 3201; Analysis 3.33).

The following secondary outcomes were not reported.

- All non-mosaic abnormalities.
- All mosaics.
- True mosaics.
- Confined mosaics.
- Reporting time (interval between sampling and result).
- Vaginal bleeding after test.
- Delivery before 33 weeks.
- Talipes (clubfoot).
- Admission to special care baby unit.
- Neonatal respiratory distress syndrome.
- Birthweight below the 10th centile.
- Birthweight below the 5th centile.

(4) Transabdominal versus transcervical CVS

Five studies with 7978 women contributed data for this comparison (Bovicelli 1986; Brambati 1991; Smidt-Jensen 1993 (Denmark); Tomassini 1988; Jackson 1992).

Primary outcomes

Compared with transabdominal CVS, total pregnancy loss and spontaneous miscarriages were higher after transcervical CVS, but this was due to the excess loss in the transcervical arm of the Smidt-Jensen 1993 (Denmark) trial. This trial reported total pregnancy loss after transcervical CVS of 12.4% compared with 7.4% after transabdominal CVS. Corresponding figures for spontaneous pregnancy loss were 8.2% and 3%. However, there was no clear difference between groups for total pregnancy loss and miscarriage rate in the other four trials (Bovicelli 1986; Brambati 1991; Tomassini 1988; Jackson 1992). Because of these differences, there was statistical heterogeneity for these two outcomes (I^2 = 72.3%). When the fixed-effect model was used to summarise the results for these two outcomes, transcervical CVS was associated with an increase in total pregnancy loss (RR 1.23, 95% CI 1.06 to 1.42) and spontaneous miscarriage (RR 1.75, 95% CI 1.33 to 2.29). However, in the presence of heterogeneity, it was prudent to apply a more conservative random-effects model. When we applied this statistical model, the differences in pregnancy loss and miscarriage between transabdominal and transcervical CVS were no longer clear (RR 1.68, 95% CI 0.79 to 3.58; Analysis 4.2 for spontaneous miscarriage, and RR 1.16, 95% CI 0.81 to 1.56; Analysis 4.1 for total pregnancy loss; very low-quality evidence).

There were many outcomes with potentially clinically important differences, but the number of events was not large enough to precisely estimate the risks. These were: all known pregnancy loss (including termination of pregnancy; 9.0% versus 7.4%; RR 1.16, 95% CI 0.81 to 1.65; studies = five; women = 7978; Analysis 4.1; *very* low-quality evidence), spontaneous miscarriage; 7.9% versus 4.5%; RR 1.68, 95% CI 0.79 to 3.58; studies = four; women = 3384; Analysis 4.2; very low-quality evidence), spontaneous miscarriage after test (pregnancy loss in women who had the test actually performed; 4.9% versus 3.9%; RR 1.23, 95% CI 0.75 to 2.04; studies = three; women = 1347; Analysis 4.3).

Secondary outcomes

Congenital anomalies were reported only in two studies with 1314 women, but the numbers were too small for meaningful comparisons (Brambati 1991; Smidt-Jensen 1993 (Denmark)).

Transcervical CVS was more likely to fail, although there was a disproportionate contribution of the data from Jackson 1992 (weight 91%). Transcervical CVS appeared to be more technically demanding, requiring more multiple insertions and causing more vaginal bleeding. As far as cytogenetic analysis was concerned, both procedures were comparable.

For technical difficulties in sampling, the transcervical CVS group had more multiple insertions (11.2% versus 4.1%; RR 2.54, 95% CI 1.47 to 4.42; studies = two; women = 1314; Analysis 4.6), and more sampling failures (RR 1.79, 95% CI 1.13 to 2.82; studies = four; women = 5231; Analysis 4.5; moderate-quality evidence). There were too few events to show a difference in laboratory failures between the two groups (1.5% versus 0.6%; RR 2.23, 95% CI 0.69 to 7.22; studies = one; women = 1194; low-quality evidence; Analysis 4.8).

There were many outcomes with potentially clinically important differences, but the number of events were not large enough to precisely estimate the risks. These were: all non-mosaic abnormalities (4.8% versus 3.9%; RR 1.23, 95% CI 0.87 to 1.75; studies = one; neonates = 2862; Analysis 4.9), true mosaics (0.7% versus 0.8%; RR 0.92, 95% CI 0.39 to 2.17; studies = one; neonates = 2862; Analysis 4.10), and confined mosaics (0.3% versus 0.4%; RR 0.85, 95% CI 0.26 to 2.77; studies = one; neonates = 2862; Analysis 4.11), amniotic leakage after test (0% versus 5%; RR 0.28, 95% CI 0.01 to 6.52; studies = one; women = 44; Analysis 4.12), vaginal bleeding after test (10.0% versus 1.6%; RR 6.93, 95% CI 0.77 to 62.83; studies = three; women = 1358; Analysis 4.13), termination of pregnancy (all; 6.6% versus 8.0%; RR 0.83, 95% CI 0.56 to 1.22; studies = two; women = 1303; Analysis 4.14), perinatal mortality (stillbirths and neonatal deaths in the first week of life; 0.3% versus 0.7%; RR 0.44, 95% CI 0.11 to 1.68; studies = one; neonates = 2037; Analysis 4.15), stillbirths (0.6% versus 0.3%; RR 1.36, 95% CI 0.11 to 17.53; number of studies = two; unborn neonates = 1227; Analysis 4.16), neonatal death (death in the first week of life; 0.1% versus 0.2%; RR 0.60, 95% CI 0.14 to 2.55; studies = two; neonates = 4845; Analysis 4.17), anomalies (all recorded; 1.4% versus 2.0%; RR 0.68, 95% CI 0.41 to 1.12; studies = two; neonates = 3622; Analysis 4.18; low-quality evidence), and talipes (clubfoot; 0.2% versus 0.07%; RR 3.21, 95% CI 0.33 to 30.80; studies = one; neonates = 2622; Analysis 4.19).

The following secondary outcomes were not reported.

- All mosaics.
- Maternal contamination.
- Known false positive after birth.
- Known false negative after birth.
- Vaginal bleeding after test.
- Antenatal hospital admission.
- Delivery before 33 weeks.
- Delivery before 37 weeks.
- All recorded deaths after viability.
- Haemangiomas (localised vascular lesions of the skin and subcutaneous tissue).
- Limb reduction defects.
- Admission to special care baby unit.
- Neonatal respiratory distress syndrome.
- Birthweight below the 10th centile.
- Birthweight below the 5th centile.

(5) Early amniocentesis (EA) versus transabdominal CVS

Four trials with 5489 women contributed data for this comparison (Leiden 1998; Nicolaides 1994 (King's); Philip 2004 (NICHD EATA); Sundberg 1997 (Copenhagen))

Primary outcomes

In the early amniocentesis group, there were more spontaneous miscarriages; 2.2% versus 1.3%; RR 1.73, 95% CI 1.15 to 2.60; studies = four; women = 5491; Analysis 5.2; moderate-quality evidence), and more spontaneous miscarriages after test (pregnancy loss in women who had the test actually performed; RR 1.71, 95% CI 1.12 to 2.61; studies = four; women = 5489; Analysis 5.3).

There was no clear difference in all known pregnancy loss (including termination of pregnancy; 3.5% versus 3.0%; RR 1.15, 95% CI 0.89 to 1.54; studies = four; women = 5491; Analysis 5.1; low-quality evidence).

Secondary outcomes

There was no clear difference in the overall incidence of anomalies in the newborn infants (RR 1.14, 95% CI 0.57 to 2.30; Analysis 5.27; very low-quality evidence). However, inter-study heterogeneity was significant for this outcome, with no obvious explanation for the observed differences between Sundberg 1997 (Copenhagen) and Leiden 1998. Both groups had specifically highlighted two types of anomalies, talipes equinovarus and haemangiomas. The incidence of talipes in the EA group was 0.9% compared with 0.1% in the CVS group (RR 3.75, 95% CI 1.42 to 9.88).

An increased number of haemangiomas after CVS, seen in Leiden 1998, was not seen in the other two studies (RR 0.75, 95% CI 0.26 to 2.20). Only Leiden 1998 reported long-term follow-up of randomised infants, and none of them had abnormal results on the Dutch version of the Denver Developmental Screening Test, when visited at home between six and nine months of age.

Transabdominal CVS appeared to be more technically demanding, with more technical difficulties during the procedure, i.e. sampling failure, multiple insertions, and the need for a second test. However, the overall incidence of these complications was low. There were no clear differences in the rate of laboratory failures or the number of women with various chromosomal abnormalities. However, the numbers were too small for any meaningful comparison between the two methods.

In Sundberg 1997 (Copenhagen), the EA samples required a mean of 9.5 days (range 5 to 19) for culturing, compared to 6.1 days (range 4 to 14) for the CVS samples. In Leiden 1998, the mean culture time in the EA group was 13.8 days for the Amniomax culture and 15.6 for the Chang culture, compared to eight days in the CVS group. In the Philip 2004 (NICHD EATA) EA, 10.3 days were needed to obtain the result (standard deviation (SD) 2.5), compared with 6.3 days (SD 3). We did not pool these results because they were not normally distributed.

There were many outcomes with potentially clinically important differences, but the number of events was not large enough to precisely estimate the risks. These were: non-compliance with allocated procedure (0.1% versus 0.7%; RR 0.25, 95% CI 0.09 to 0.72; studies = four; women = 5566; Analysis 5.4), sampling failure (0.5% versus 0.8%; RR 0.58, 95% CI 0.24 to 1.38; studies = four; women = 5566; Analysis 5.5; low-quality evidence), multiple insertions (1.1% versus 2.5%; RR 0.45, 95% CI 0.21 to 0.95; studies = three; women = 4445; Analysis 5.6), second test performed (0.8% versus 1.4%; RR 0.63, 95% CI 0.28 to 1.43; studies = four; women = 5566; Analysis 5.7), laboratory failure (0.4% versus 0.6%; RR 0.74, 95% CI 0.34 to 1.63; studies = four; women = 5566; Analysis 5.8; low-quality evidence), all non-mosaic abnormalities (1.7% versus 1.7%; RR 0.95, 95% CI 0.47 to 1.90; studies = four; neonates = 5566; Analysis 5.9), true mosaics (0.1% versus 0.2%; RR 0.47, 95% CI 0.10 to 2.20; studies = three; neonates = 5451; Analysis 5.10), maternal contamination (0.4% versus 0.1%; RR 1.92, 95% CI 0.02 to 162.80; studies = two; women = 4330; Analysis 5.12), known false positive after birth (0.0% versus 0.3%; RR 0.36, 95% CI 0.02 to 8.73; studies = two; neonates = 670; Analysis 5.13), known false negative after birth (no events reported; studies = one; neonates = 555; Analysis 5.14; low-quality evidence), vaginal bleeding after test (1.5% versus 2.2%; RR 0.69, 95% CI 0.42 to 1.12; studies = three; women = 4934; Analysis 5.18), amniotic leakage after test (9.0% versus 3.6%; RR 3.35, 95% CI 0.37 to 30.09; studies = three; women = 4934; Analysis 5.17), vaginal bleeding after 20 weeks (0.7% versus 1.0%; RR 0.71, 95% CI 0.35 to 1.43; studies = one; women = 3698; Analysis 5.16), pre-labour ruptured membranes before 28 weeks (0.8% versus 1.7%; RR 0.50, 95% CI 0.27 to 0.92; studies = one; women = 3698; Analysis 5.19), delivery before 37 weeks (5.5% versus 4.8%; RR 1.16, 95% CI 0.78 to 1.74; studies = three; women = 1755; Analysis 5.20), delivery before 33 weeks (0.4% versus 0.7%; RR 0.50, 95% CI 0.09 to 2.73; studies = one; women = 1121; Analysis 5.21; low-quality evidence), termination of pregnancy (all; 0.9% versus 1.2%; RR 0.74, 95% CI 0.45 to 1.25; studies = four; women = 5489; Analysis 5.22), perinatal mortality (stillbirths and neonatal deaths in the first week of life; 0.6% versus 0.5%; RR 1.10, 95% CI 0.53 to 2.28; studies = four; neonates = 5428; Analysis 5.23), stillbirths (0.5% versus 0.5%; RR 1.11, 95% CI 0.52 to 2.36; studies = four; neonates = 5428; Analysis 5.24), neonatal death (death in the first week of life; 0.04% versus 0.1%; RR 0.41, 95% CI 0.05 to 3.11; studies = four; neonates = 5455; Analysis 5.25), all recorded deaths after viability (0.3% versus 0.3%; RR 1.18, 95% CI 0.43 to 3.24; studies = four; neonates = 5453; Analysis 5.26), anomalies (all recorded; 3.0% versus 2.5%; RR 1.14, 95% CI 0.57 to 2.30; studies = four; neonates = 5305; Analysis 5.27; very low-quality evidence), talipes (clubfoot; 0.9% versus 0.2%; RR 3.75, 95% CI 1.42 to 9.88; studies = four; neonates = 5305; Analysis 5.28), haemangiomas (localised vascular lesions of the skin and subcutaneous tissue; 4.5% versus 5.2%; RR 0.75, 95% CI 0.26 to 2.20; studies = four; neonates = 5305; Analysis 5.29), neonatal respiratory distress syndrome (defined by authors; 0.4% versus 0.4%; RR 0.91, 95% CI 0.21 to 3.98; studies = four; neonates = 4725; Analysis 5.30), birthweight below the 10th centile (6.6% versus 7.9%; RR 0.84, 95% CI 0.66 to 1.06; studies = one; neonates = 3618; Analysis 5.31), birthweight below the 5th centile (2.9% versus 2.8%; RR 0.66, 95% CI 0.05 to 9.38; studies = two; neonates = 629; Analysis 5.32).

The following secondary outcomes were not reported.

- All mosaics.
- Confined mosaics.
- Antenatal hospital admission.
- Limb reduction defects.
- Admission to special care baby unit.

(6) Ultrasound-guided amniocentesis

Nolan 1981 (223 women) evaluated the type of ultrasound-assisted procedure that is currently considered obsolete (i.e. this was not an ultrasound-guided procedure in the true meaning of this term). There were no clear differences in the reported outcomes, but the study was too small to assess the true impact of the placental localisation by ultrasound before the needle insertion.

Primary outcomes

There were potentially clinically important differences in all known pregnancy loss (including termination of pregnancy; 0.0% versus 0.9%; RR 0.33, 95% CI 0.01 to 8.02; studies = one; women = 223; Analysis 6.1), spontaneous miscarriage; 0.0% versus 0.9%; RR 0.33, 95% CI 0.01 to 8.02; studies = one; women = 223; Analysis 6.2), spontaneous miscarriage after test (pregnancy loss in women who had the test actually performed; 0.0% versus 0.9%; RR 0.33, 95% CI 0.01 to 8.02; studies = one; women = 223; Analysis 6.3), but the number of events were not large enough to precisely estimate the risks.

Secondary outcomes

For outcomes related to technical difficulties in sampling, there was more sampling failure (4.5% versus 0.0%; RR 10.90, 95% CI 4.5 to 0.0; studies = one; women = 223; Analysis 6.4) and fewer multiple insertions (18.8% versus 27.9%; RR 0.67, 95% CI 0.41 to 1.09; studies = one; women = 223; Analysis 6.5) in the ultrasound group.

The following secondary outcomes were not reported.

- Non-compliance with allocated procedure.
- Second test performed.
- Laboratory failure.
- All non-mosaic abnormalities.
- All mosaics.
- True mosaics.
- Confined mosaics.
- Maternal contamination.
- Known false positive after birth.
- Known false negative after birth.
- Vaginal bleeding after test.
- Amniotic leakage after test.
- Vaginal bleeding after 20 weeks.
- pre-labour ruptured membranes before 28 weeks.
- Antenatal hospital admission.
- Delivery before 37 weeks.
- Delivery before 33 weeks.
- Termination of pregnancy (all) 'included in all known pregnancy loss'.
- Perinatal mortality (stillbirths and neonatal deaths in the first week of life).
- Stillbirths.
- Neonatal death (death in the first week of life).
- All recorded deaths after viability.
- Anomalies (all recorded).

- Talipes (clubfoot).
- Haemangiomas (localised vascular lesions of the skin and subcutaneous tissue).
- Limb reduction defects.
- Admission to special care baby unit.
- Neonatal respiratory distress syndrome.
- Birthweight below the 10th centile.
- Birthweight below the 5th centile.

DISCUSSION

Summary of main results

The only estimate from randomised trials of an 'excess' risk after second trimester amniocentesis came from Tabor 1986. In a low-risk population with a background pregnancy loss of around 2%, a mid-trimester amniocentesis increased this risk by another 1%. It is important to stress that the estimate is relatively imprecise, with 95% confidence intervals (CI) for absolute risk difference ranging from 0% to 2%.

Women who request early diagnostic procedures (e.g. because of religious or personal prohibitions on later pregnancy termination, or because of a very high risk of fetal abnormalities) should be counselled about the relative risks of the various options. There is now enough moderate to high-quality evidence to conclude that early amniocentesis is inferior to second trimester amniocentesis, given the increased risk of miscarriages and congential anomalies (talipes).

The benefits of earlier diagnosis by chorionic villus sampling (CVS) must also be set against possible higher risks of pregnancy loss and diagnostic inaccuracies when compared with second trimester amniocentesis. Unfortunately, the data related to the risk of pregnancy loss following both transabdominal and transcervical CVS and amniocentesis were inconsistent. As far as CVS method (route) is concerned, from relatively limited randomised evidence, transabdominal CVS appeared to be safer than the transcervical route.

The question about diagnostic accuracy of prenatal testing remains unanswered, and the hypothesis that both CVS and amniocentesis are equally accurate remains untested, because of incomplete follow-up.

Another area of concern is the possibility of a causal relationship between some fetal abnormalities and invasive procedures in early pregnancy. The difference in the incidence of congenital anomalies observed when early amniocentesis was compared to transabdominal CVS did not reach the conventional level for statistical significance, but it did when early amniocentesis was compared with second trimester amniocentesis.

Overall completeness and applicability of evidence

Despite relatively large numbers of randomised women (4606) in Tabor 1986, such an increase in total pregnancy loss did not reach statistical difference, with CIs for an excess pregnancy loss ranging from almost 0 to 2%. How robust are these figures, and should they be used for routine counselling? It is unlikely that a trial of similar size and quality will ever be repeated. Therefore, in the absence of other randomised data, written and oral information for women considering second trimester amniocentesis has included the data from Tabor 1986. However, several systematis reviews that added observational data to the randomised evidence have been published since, showing significantly lower complication rates (see Agreements and disagreements with other studies or reviews).

Second trimester amniocentesis was consistently safer than transcervical CVS, whilst Smidt-Jensen 1993 (Denmark) showed no clinically significant difference in the pregnancy loss between transabdominal CVS and second trimester amniocentesis. Therefore, one would expect a clear benefit of transabdominal CVS in the 'head to head' comparisons with transcervical CVS. Unfortunately, the data were quite heterogeneous; for example, Smidt-Jensen 1993 (Denmark) showed expected benefits of transabdominal CVS, but other trials did not. It is likely that operator skill and preferences played an important role in these studies.

It would be unrealistic to expect that any given operator would be equally skilled and experienced in all three methods. The question, whether any added risks of early procedures, transcervical CVS in particular, disappear in the hands of skilled operators remains one of the main controversies of fetal medicine. In most included trials, the operators were required to perform at least 20 successful early procedures in order to participate. Some performed thousands successfully, and therefore, undoubtedly, the experience between operators varied. Interestingly, in the MRC 1991 trial, there was no clear evidence that individual operators' performance improved with more experience over the course of the study.

Somewhat unexpectedly, the preliminary data from the Nicolaides 1994 (King's) and Leiden 1998 trials suggested an important increase in pregnancy loss following early amniocentesis, both before and after fetal viability. However, pooled data from the final reports of these two trials and Sundberg 1997 (Copenhagen) were not conclusive. In order to test the hypothesis that the total pregnancy loss after early amniocentesis is indeed 0.5% higher compared with CVS, around 40,000 women would need to be recruited (power 80%, confidence level 95%). Such a trial is likely to be considered unethical, given the strong possibility of causal relationship between early amniocentesis and talipes (*see* below).

The observation that transabdominal CVS appeared safer than transcervical CVS was heavily influenced by the data from Smidt-Jensen 1993 (Denmark). Increase in pregnancy loss following transcervical procedure was not replicated in four other direct comparisons between transcervical and transabdominal procedures (Bovicelli 1986; Brambati 1991; Tomassini 1988; Jackson 1992). The transcervical approach required multiple insertions more frequently and caused vaginal bleeding in approximately 10% of cases. The subgroup analysis from Smidt-Jensen 1993 (Denmark) showed no differential effect on the pregnancy loss between transabdominal CVS and mid-trimester amniocentesis. It would be reassuring if the results achieved by Smidt-Jensen and colleagues could be replicated by other centres (71% of all procedures in the Smidt-Jensen 1993 (Denmark) trial were performed by Smidt-Jensen himself). The results of the systematic review of observational studies were broadly consistent with the randomised data (Mujezinovic 2007).

We acknowledge the ethical and potential medico-legal problems in trying to obtain adequate cytogenetic follow-up on all randomised women. A higher incidence of abnormal karyotypes is to be expected in the CVS group, because of possible

spontaneous loss of pregnancies with abnormal karyotype that occur between randomisation and a mid-trimester amniocentesis group. With this proviso, the available data suggested that accurate diagnosis was more likely following second trimester amniocentesis. Abnormalities confined to the placenta (placental mosaics) pose a particular problem for women who opt for CVS. Although the absolute numbers were small, both known false positive and known false negative after birth results have such a devastating effect that observed differences should not be ignored.

An increased incidence of talipes equinovarus after early amniocentesis was specifically highlighted, with 24/2612 cases in the early amniocentesis group compared to only 5/2693 cases in the CVS group (RR 3.75, 95% CI 1.42 to 9.88; studies = four; participants = 5305). Early amniocentesis enthusiasts may argue that the possibility of ascertainment bias needs to be borne in mind when the data from unblinded trials are interpreted. However, it would be virtually impossible to blind women and clinicians to the type of invasive prenatal test actually carried out, because the type and handling of the obtained tissue (amniotic fluid or chorionic villi) are distinctly different. Under those circumstances, one may look harder for certain type of anomalies, i.e. talipes, in babies known to have early amniocentesis, and not record them when causation is unlikely (after CVS). In our view, the above data are compelling, and every effort should be made to ensure that amniocentesis is not performed before 15 weeks' gestation.

Quality of the evidence

We judged that overall, the included studies were at low risk of bias, though specific details of the randomisation, allocation concealment, and blinding of outcome assessment were unavailable in several cases. Though it was not feasible to blind included studies due to the nature of the comparisons, Brambati 1991 was judged to be at high risk of performance bias, as the proportion of cases where the operator deviated from the allocated procedure increased significantly during the study (4.6, 9.7, and 15.5% during years one to three), with deviations weighted towards the transcervical CVS arm. Borrell 1999 was deemed to be at high risk of attrition bias due to a large and unbalanced dropout rate (assigned procedure performed in 681/1011 women). Nicolaides 1994 (King's) was also judged to be at high risk of bias, partly due to the randomisation sequence described, with the selection of one of two non-sequentially numbered envelopes, and also because adverse publicity surrounding CVS caused the trial to stop prematurely.

We used GRADEpro software to grade a set of primary and secondary outcomes, where data were available. These outcomes were judged to be important for clinical decision making, and were graded for five of the comparisons. Evidence ranged from high to very low-quality. Evidence was downgraded where CIs were wide and crossed the line of nil effect, when study design limitations, such as randomisation methods, were not specified, and when heterogeneity was high between the studies.

For second trimester amniocentesis compared to control, we found high-quality evidence for laboratory failure, and spontaneous miscarriage, and moderate-quality evidence for all known pregnancy losses and anomalies.

For early compared to second trimester amniocentesis, we found high-quality evidence for laboratory failure, all known pregnancy losses, and all anomalies. We assessed the evidence to be moderate for spontaneous miscarriages, sampling failure, and false negative chromosomal diagnosis after birth.

For transabdominal CVS compared to amniocentesis, we judged the evidence to be low quality for spontaneous miscarriage, all known pregnancy losses, and perinatal deaths.

For transcervical compared to transabdominal CVS, we found moderate-quality evidence for sampling failure, and low-quality evidence for laboratory failure, and anomalies, and very low-quality for spontaneous miscarriage, and all known pregnancy losses.

For early amniocentesis compared to transabdominal CVS, we found moderate-quality evidence for spontaneous miscarriage, low-quality evidence for sampling and laboratory failure, all known pregnancy losses, delivery before 33 weeks' gestation, and known false negative after birth results. We only found very low-quality evidence for anomalies.

Potential biases in the review process

There is the potential to introduce bias at each stage of any review process, and necessary steps were taken to minimise specific risks. A minimum of two authors independently reviewed each study, and disagreements were resolved by discussion with a third author. At least two authors independently conducted the data extraction and 'risk of bias assessments' of each included study. 'Risk of bias' assessments will always carry an element of subjectivity, based on judgements, and for this reason cannot be considered completely objective. None of the authors were involved in studies included in this review.

Agreements and disagreements with other studies or reviews

We identified four non-Cochrane systematic reviews of observational studies, two assessing risks in singleton pregnancies, and two in twin pregnancies (Agarwal 2012; Akolekar 2015; Mujezinovic 2007; Vink 2012). These reviews of observational data merit discussion, as they influenced the interpretation of procedure-related loss rates for amniocentesis and CVS, and provided some information about the magnitude of procedure-related risks in twin pregnancies (Table 1; Table 2).

Mujezinovic 2007 reviewed observational studies that reported complications for transabdominal CVS performed between 10 and 14 weeks' gestation, and genetic amniocentesis performed after 14 weeks' gestation. This review included studies published from January 1995, and reporting data for 100 or more participants. The pooled pregnancy loss rates within 14 days of the procedure were 0.7 (95% CI 0.3 to 1.4) and 0.6 (95% CI 0.5 to 0.7). A more recent systematic review and meta-analysis by Akolekar 2015 reviewed observational studies published between 2000 and 2014 that reported procedure-related complications for both CVS and amniocentesis. They only included studies with more than a thousand procedures reported. In order to minimise risk of bias from smaller studies, they used a random-effects model to calculate risks. This meta-analysis described weighted pooled procedure-related risks of miscarriage for procedures performed prior to 24 weeks' gestation of 0.22% for CVS and 0.11% for amniocentesis, which was much lower than previously quoted. There was no clear difference between the procedure-related risk

of miscarriage for either procedure, or to the background risk of miscarriage. The authors of these reviews highlighted concerns with the difficulty of adequate control groups. The meta-analysis in Akolekar 2015 also found significant heterogeneity. However, it is likely that the estimates that only come from RCTs performed many years ago overestimate the risks (Table 1; Table 2).

Agarwal 2012 completed a systematic review of observational studies in twin pregnancies to assess the risks of CVS performed between 9 and 14 weeks' gestation, and genetic amniocentesis performed between 14 and 22 weeks' gestation. They included study reports published between January 1990 and May 2011. The overall pregnancy loss rates were 3.84% (95% CI 2.48 to 5.47) for CVS, and 3.07% (95% CI 1.83 to 4.61) for amniocentesis. The authors described an excess pregnancy loss of approximately 1% above the background rate for both procedures. There were no clear differences in pregnancy loss rates between transabdominal or transcervical CVS, or amniocentesis performed with either single or double entry technique. Vink 2012 also conducted a systematic review of observational studies that assessed pregnancy loss rates in women with twin pregnancies undergoing genetic amniocentesis, but included a broader time-frame for publications (from January 1970 until December 2010). They used random-effects models to pool procedure-related loss rates. The authors commented on significant heterogeneity in the literature, but were able to report a pooled pregnancy loss rate before 24 weeks' gestation of 3.5% (95% CI 2.6 to 4.7). Taking into account the pregnancy loss rates before 24 weeks reported by Agarwal 2012 and Vink 2012, the true pregnancy loss rate at this gestation is likely to lie between 2.5 to 3.5% (Table 2).

Observational data have suggested an increased incidence of haemangiomas in infants born after their mothers underwent CVS (Burton 1995). Similarly to the risk of oromandibular or limb hypogenesis, and isolated limb disruption defects, the association with CVS remains controversial (NICHHD 1993). Plausible mechanisms include transient fetal hypoperfusion secondary to bleeding into the sampling site, the release of vasoactive substances from the placenta causing vasoconstriction or haemorrhage in the fetus, or a combination. It is reassuring that there were no reported oromandibular or limb hypoplasias in the three trials, which may reflect the fact that all procedures were done after nine weeks' gestation.

AUTHORS' CONCLUSIONS

Implications for practice

Parents considering prenatal diagnosis must be fully informed about the risks and benefits of the alternative procedures before they make a choice. Second trimester amniocentesis is safer than early amniocentesis or transcervical chorion villus sampling (CVS). If earlier diagnosis is required, transabdominal CVS is preferable to early amniocentesis or transcervical CVS.

Although CVS technique is more likely to result in an ambiguous result, the diagnostic accuracy of different methods could not be assessed adequately because of incomplete karyotype data in most studies.

Implications for research

New methods of prenatal diagnosis should be rigorously evaluated before deciding whether they should be introduced into clinical practice. Measures of outcome must include total pregnancy loss (antenatal and neonatal), detailed description of anomalies, diagnostic accuracy, and women's views of the alternative procedures. Ascertainment bias should be reduced as much as possible, i.e. neonatal assessors should be blinded to the allocated procedure.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Ammala 1993 (MRC Finland)

Methods	Consecutively-numbered sealed envelopes
Participants	800 women in early pregnancy requesting prenatal diagnosis
Interventions	4 operators performed all procedures - TC CVS with Portex cannula or AC at 16 weeks under ultrasound guidance
Outcomes	Pregnancy outcome, abnormal karyotype, antenatal complications, and diagnostic accuracy
Notes	This study was part of the international MRC trial Dates of study: not reported Setting: hospital in Helsinki, Finland Funding: not reported Conflict of interest: not reported

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Block randomisation

Ammala 1993 (MRC Finland) *(Continued)*

Allocation concealment (selection bias)	Low risk	Allocation concealed in consecutively numbered sealed envelopes
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	Participants appropriately accounted for, intention-to-treat analysis
Selective reporting (reporting bias)	Low risk	Specified outcomes reported
Other bias	Low risk	Comparable groups

Borrell 1999

Methods	Random telephone allocation using a table of random numbers
Participants	Women requesting fetal karyotyping on the basis of advanced maternal age prior to 12th completed week Exclusions included: multiple pregnancies, menstrual gestational age greater than 11 plus 6 weeks, or an indication for cytogenetic analysis other than advanced maternal age 503 randomised to CVS group and 508 to the AC group
Interventions	TC CVS performed from 9th to 13th week of pregnancy using round tipped curved steel forceps after initial ultrasound scan. Procedure performed under direct ultrasound guidance. AC was performed from the 15th to 18th week of pregnancy using 22 G needle under direct ultrasound guidance.
Outcomes	Diagnostic success and fetal loss rate
Notes	Trial prematurely discontinued when second trimester serum biochemistry screening was introduced. 110 women miscarried before the assigned procedure; 68 in the CVS group and 42 in the AC group. In total, the assigned procedure was performed in only 67% of randomised women (681/1011). Dates of study: not reported Setting: hospital in Barcelona, Spain Funding: not reported in translation Conflict of interest: not reported in translation

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomised using a table of random numbers in blocks of 16

Borrell 1999 *(Continued)*

Allocation concealment (selection bias)	Low risk	Allocated via telephone
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	High risk	The assigned procedure was performed in 681/1011 women. A large and uneven dropout rate may be a source of significant bias and data from this trial have to be interpreted with caution.
Selective reporting (reporting bias)	Low risk	Outcomes appropriately reported
Other bias	Unclear risk	Trial prematurely discontinued when second trimester serum biochemistry screening was introduced. We were unsure of the impact of insufficient recruitment to fulfil sample size calculation.

Bovicelli 1986

Methods	Randomly assigned - method not described
Participants	Inclusion criteria: gestational age 9 to 13 weeks, viable embryo with an intact sac
Interventions	TC performed under direct ultrasound guidance. 16 G cannula passed via the cervix to chorion frondosum and villi aspirated with suction. TA CVS was performed using continuous ultrasound guidance and an 18 G needle passed to reach the border of the chorion frondosum. A 20 G needle was then passed through this first needle and villi aspirated.
Outcomes	Technical difficulty, fetal loss rate, and speed of procedure
Notes	Dates of study: not reported Setting: hospital in Bologna, Italy Funding: not reported Conflict of interest: not reported

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Method not described
Allocation concealment (selection bias)	Unclear risk	Method not described

Bovicelli 1986 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Data appeared accounted for (reported in letter format only)
Selective reporting (reporting bias)	Unclear risk	Outcomes reported in letter format
Other bias	Low risk	None identified

Brambati 1991

Methods	Randomisation by telephone
Participants	Women aged between 19 and 48 years attending for first trimester fetal diagnosis of genetic diseases. Indications for fetal diagnosis included chromosomal aberration, sex determination for X-linked diseases, metabolic diseases, DNA analysis for haemoglobinopathies and haemophilias. Gestational age between 8 and 12 weeks. Exclusion criteria: multiple pregnancy, vaginal infection, pending cerclage, vaginal bleeding, and placenta inaccessible via either cervical canal or abdominal wall.
Interventions	TC and TA CVS were performed using a 20 G needle; no more than 2 cannula or needle insertions used in 1 session.
Outcomes	Technical difficulty and quantity of tissue obtained, along with pregnancy outcome
Notes	Dates: March 1986 - July 1988 Setting: hospital in Milan, Italy Funding: partially supported by WHO Hereditary Disease Programme Conflict of interest: not reported

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Unstratified block randomisation by telephone
Allocation concealment (selection bias)	Low risk	Allocation list held at the trial service unit
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not feasible to blind participants or personnel due to the nature of the comparison. The proportion of cases where the operator deviated from the allocated procedure increased during the study (4.6%, 9.7%,and 15.5% during years 1

Brambati 1991 *(Continued)*

		to 3). Majority of deviations were in the TC CVS arm, where deviations were 6%, 16%, and 27% in years 1 to 3, respectively.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	All participants appropriately accounted for, intention-to-treat analysis
Selective reporting (reporting bias)	Low risk	All specified outcomes reported
Other bias	Low risk	Balanced groups

Canada 1989

Methods	Central randomisation (unknown), and stratified according to age 35 to 38, ≥ 39, and centre.
Participants	Participants from 12 centres in Canada. Eligible women - aged 35 years or older at time of delivery, or those referred for fetal chromosome analysis, less than 12 weeks' gestation, viable singleton intrauterine pregnancy confirmed by ultrasound Women excluded if dead or disorganized embryo, multiple pregnancy, Rh isoimmunisation, untreated cervical infection, or gestation greater than 12 weeks. 2787 women randomised 396 ineligible following randomisation 1391 randomised to CVS (200 ineligible) 1396 randomised to AC (196 ineligible)
Interventions	TC vs second trimester AC
Outcomes	Technical difficulties, abnormal karyotype, pregnancy complications, perinatal loss, neonatal complications, and cytogenetic accuracy
Notes	Dates of study: October 1987 - September 1988 Setting: multiple centres in Canada Funding: Canadian Medical Research Council Conflict of interest: not reported

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Method not described
Allocation concealment (selection bias)	Low risk	Central randomisation by telephone

Canada 1989 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Outcomes not reported for 72 (5.3%) participants randomised to CVS and 90 (6.6%) randomised to AC. 22 and 30 lost to follow-up in CVS and AC groups respectively.
Selective reporting (reporting bias)	Unclear risk	Outcomes not reported for 72 (5.3%) participants randomised to CVS and 90 (6.6%) randomised to AC.
Other bias	Low risk	Balanced groups

CEMAT 1998

Methods	Telephone randomisation, random allocation list computer generated
Participants	4368 participants in 12 centres. Inclusion criteria: prenatal diagnosis due to maternal age, newborn baby with a chromosomal abnormality, viable fetus with a CRL of 20 mm to 50 mm on ultrasound, and consent to enter the trial Exclusion criteria: previous open neural tube defect detected by prenatal diagnosis, molecular or biochemical disorders found on prenatal tests, non viable fetus, multiple pregnancy, failed CVS, fetal anomaly or oligohydramnios, active vaginal bleeding, alloimmunised patient, recurrent unexplained miscarriages, intrauterine contraceptive device in utero, previous CEMAT trial randomisation
Interventions	Both groups underwent detailed fetal anomaly ultrasound examination at 15 and 20 weeks. EAC group had AC performed between 11 and 12 gestational weeks, and second trimester between 15 and 16 weeks. All AC were performed under direct ultrasound guidance using 22 G, 9 cm, or 14 cm needles.
Outcomes	Pregnancy outcome, congenital anomalies, abnormal karyotype, and technical difficulty
Notes	Dates of study: enrolment from July 1994 to December 1996, follow-up finished in 1997 Setting: 12 participating centres in Canada Funding: MRC of Canada Clinical Trials Committee Conflict of interest: not reported

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer generated randomisation
Allocation concealment (selection bias)	Low risk	Centralised allocation via telephone

CEMAT 1998 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	Participants appropriately accounted for, intention-to-treat analysis
Selective reporting (reporting bias)	Low risk	All specified outcomes reported
Other bias	Low risk	Comparable groups

Jackson 1992

Methods	Random assignment
Participants	3998 patients recruited in 8 US collaborating centres. Inclusion criteria: favourable placental position allowing both procedures to be performed, gestational age between 49 and 90 days Exclusion criteria: active genital herpes, active vaginal bleeding or cervical polyps 1190 randomised to TC CVS and 1163 to TA CVS
Interventions	TA or TC CVS. TC performed with a plastic catheter, and TA with an 18 to 22 G spinal needle
Outcomes	Sampling success, pregnancy outcome
Notes	Initial cohort of 2353 women, who delivered before July 1 1989 Dates of study: April 1987 to September 1989 Setting: hospital in US Funding: not reported Conflict of interest: not reported

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomisation method not described
Allocation concealment (selection bias)	Unclear risk	Not specified

Jackson 1992 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Outcome not reported for women where sampling was not attempted (3.2%). For the majority of important clinical outcomes including type of pregnancy loss, intention-to-treat analysis was not feasible because data were presented only for women with genetically normal pregnancies (91.5%).
Selective reporting (reporting bias)	Unclear risk	Outcomes not reported for women where sampling was not attempted (3.2%)
Other bias	Low risk	None identified

Leiden 1998

Methods	EA vs TA CVS. Women eligible were given the choice for randomisation or to decide the method of prenatal diagnosis themselves. Allocation was performed using sequentially numbered envelopes.
Participants	Women requesting prenatal diagnosis due to age related risk. 212 women were recruited, 115 agreed to be randomised; 70 chose EA and 25 CVS. 2 women did not participate because fetal death was diagnosed before any intervention.
Interventions	TA CVS was performed using a 20 G needle. AC was performed using a 22 G needle: 11 mL of amniotic fluid was aspirated, the first mL being discarded.
Outcomes	Technical difficulties, abnormal karyotype, pregnancy complications, perinatal loss, neonatal complications, Dutch version of Denver Developmental Screening Test at 6 to 9 months.
Notes	Study stopped after 18 months following advice of the institutional ethics committee due to a higher incidence of fetal loss in the EA group. Dates of study: two years, dates not specified Setting: Leiden University Hospital, the Netherlands Funding: not reported Conflict of interest: not reported

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Method of randomisation not specified
Allocation concealment (selection bias)	Low risk	Sequentially numbered envelopes

Leiden 1998 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	All data accounted for
Selective reporting (reporting bias)	Low risk	Specified outcomes reported
Other bias	Unclear risk	The number of women who did not receive the intervention according to allocation was not evenly distributed between the groups.

MRC 1991

Methods	Central telephone randomisation. Random allocation in balanced blocks and stratified by centre. Finland - consecutively numbered, sealed, opaque envelopes.
Participants	3248 recruited from 31 centres in Europe (21 in the UK, 4 in Italy, 2 in the Netherlands, and 1 in Finland, Denmark, Switzerland, and Germany). Prenatal diagnosis due to maternal age. Other indications were anxiety and previously affected child with chromosome anomaly. Centres eligible if each participating obstetrician had performed at least 30 procedures with > 10 mg of tissue in 23 out of 25 most recent cases. 1609 randomised to CVS and 1592 to AC.
Interventions	First trimester CVS TC or TA approach vs second trimester AC
Outcomes	Pregnancy outcome, abnormal karyotype, antenatal complications, and diagnostic accuracy
Notes	Dates of study: recruited from 1985-1989 Setting: 31 centres in Europe (21 in the UK, 4 in Italy, 2 in the Netherlands, and 1 in Finland, Denmark, Switzerland, and Germany) Funding: grant from Department of Health, UK Conflict of interest: not reported

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Block randomisation
Allocation concealment (selection bias)	Low risk	Central allocation by telephone. In Finland, consecutively numbered, sealed, opaque envelopes were used.
Blinding of participants and personnel (performance bias)	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and

MRC 1991 *(Continued)*

All outcomes		recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Data collected by clinical team, unclear if laboratory staff blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	Participants appropriately accounted for
Selective reporting (reporting bias)	Low risk	Specified outcomes reported
Other bias	Low risk	Comparable groups

Nicolaides 1994 (King's)

Methods	Sealed opaque envelope containing a card for 1 of the procedures. Not sequentially numbered envelopes.
Participants	Median age 38 years range (22 to 46). Inclusion criteria: ultrasonographic evidence of a viable fetus at 10 to 13 weeks 6 days' gestation (minimum CRL = 38 mm) and maternal request for karyotyping due to advanced maternal age, anxiety, or family history of chromosomal abnormality. Exclusions: increased nuchal translucency, missed abortion, multiple pregnancy, major fetal abnormality, intrauterine device, multiple fibroids, or large placental haemorrhage. EA was performed in 840 women (278 after randomisation), and CVS in 652 women (277 after randomisation).
Interventions	EA vs CVS. Both procedures being carried out by Professor Nicolaides, or under his direct supervision. A freehand technique and a 20 G needle was used for both EA and CVS. No local anaesthesia, prophylactic antibiotics, or bed rest EA: 11 mL of fluid aspirated, first 1 mL discarded CVS: 6 to 10 mL of tissue aspirated manually through a 20 mL syringe
Outcomes	Technical difficulties, abnormal karyotype, pregnancy complications, perinatal loss, and maternal complications
Notes	Aimed to recruit 4400 women. However, by March 1993 recruitment collapsed because of widespread publicity that CVS could cause fetal limb abnormalities, and was associated with a high risk of spontaneous abortion, and that non-invasive screening by ultrasonography and maternal serum biochemistry could provide sufficient reassurance to avoid invasive testing. Dates of study: January 1990 to March 1993 Setting: hospital in London Funding: not reported Conflict of interest: not reported

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**

Nicolaides 1994 (King's) *(Continued)*

Random sequence generation (selection bias)	High risk	Randomised with a choice of 2 non-sequentially numbered envelopes selected by the participant
Allocation concealment (selection bias)	Low risk	Allocation concealed in a sealed opaque envelope
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcome data available for all but 1 participant
Selective reporting (reporting bias)	Low risk	All outcomes reported
Other bias	High risk	Trial stopped due to adverse publicity regarding risks associated with CVS

Nolan 1981

Methods	Random allocation (method unknown)
Participants	223 women randomised
Interventions	Mid-trimester AC with or without 'the obstetrician having the benefit of ultrasound results'. It appeared that ultrasound was used to locate the placenta, i.e. the procedure was not performed under direct ultrasound guidance.
Outcomes	Number of taps, bloody taps
Notes	Dates of study: not clear Setting: hospital in US Funding: not reported Conflict of interest: not reported

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Sequence generation not described
Allocation concealment (selection bias)	Unclear risk	Not specified

Nolan 1981 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	Participants appropriately accounted for
Selective reporting (reporting bias)	Low risk	Specified outcomes reported
Other bias	Low risk	Comparable groups

Philip 2004 (NICHD EATA)

Methods	Telephone randomisation interactive voice response computer-based system
Participants	14 clinical centres. Inclusion criteria: age of mother more than 34 years, previous affected child, positive screening test. Exclusion criteria: multiple pregnancy, familiar chromosome rearrangements, inherited enzyme disorders, serious maternal illnesses (insulin-dependent diabetes, severe hypertension, HIV), bleeding equal menstruation, IUD in situ, oligohydramnios, recognised fetal abnormalities. Total number of patients = 3775 (CVS group = 1914 and EAC group = 1861)
Interventions	EAC group: 22 G spinal needle, 1 mL for each week CVS - single (19 to 20 G) and double needle technique (18 to 20 G). Larger guide needle to the margin of the chorion
Outcomes	Primary outcome: fetal loss at less than 28 weeks. Secondary outcome: all fetal loss, all neonatal death, oligohydramnios, gestational age at the delivery, IUGR, respiratory distress syndrome, limb reduction defects, talipes equinovarus, other congenital anomalies
Notes	Dates of study: January 1997 - December 2001 Setting: hospitals in Denmark, US, and Canada Funding: The principal funding source was the U.S. National Institute of Child Health and Human Development (NICHD), which approved the design of the trial. Neither the NICHD nor the Centre for Evaluation and Health Technology Assessment of the Danish National Board of Health (which funded continuation of enrolment in 2001) had any role in data collection, analysis, or interpretation of the data. Conflict of interest: Not reported

Risk of bias

Bias	Authors' judgement	Support for judgement

Philip 2004 (NICHD EATA) *(Continued)*

Random sequence generation (selection bias)	Low risk	Computer-based randomisation (urn method), stratified by clinical centre
Allocation concealment (selection bias)	Low risk	Centrally allocated, via telephone using an interactive voice response system
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	99.9% of participants randomised were followed through to delivery
Selective reporting (reporting bias)	Low risk	Specified outcomes reported
Other bias	Low risk	Comparable groups

Smidt-Jensen 1993 (Denmark)

Methods	3-way randomisation of low-risk women (TA vs TC vs AC). A 2-way randomisation of high-risk women (TA vs TC). Central randomisation (unknown) with stratification for genetic risk
Participants	2 centres in Denmark from 1985 to 1990. Eligible low-risk women: age > 34, or father > 49, history of, or anxiety about chromosomal abnormality, > 3 spontaneous miscarriages with viable fetus at 9 to 11 weeks Eligible high-risk women: history of translocation, late termination, or fetus at risk of metabolic disorder with a viable fetus at 9 to 11 weeks Exclusions: active bleeding, intrauterine device, genital infection, severe mental illness, use of teratogenic drugs, history of neural tube defects, and discrepant dating
Interventions	CVS vs second trimester AC TA CVS vs second trimester AC TC CVS vs second trimester AC TC CVS vs TA CVS
Outcomes	Pregnancy outcome, antenatal complications, and diagnostic accuracy
Notes	Dates of study: August 1985 to October 1990 Setting: 2 hospitals in Denmark Funding: This study was supported by grants from Gangstedfonden, Egmont H. Petersens Fond, Fru Lily Benthine Lunds Fond, Rosalie Petersens Fond, Meda A/S, Bruel og Kjaer, S&W Fondet, Tuborgfondet, Unisis Corp., Winterthur-borgen Legatet, Hafuia Fonden, Kromosomforskningsfonden, and the US National Institute of Child Health and Human Development. Conflict of interest: not reported

Smidt-Jensen 1993 (Denmark) *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not specified
Allocation concealment (selection bias)	Low risk	Central allocation via telephone
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	All data accounted for
Selective reporting (reporting bias)	Low risk	Specified outcomes reported
Other bias	Low risk	Comparable groups

Sundberg 1997 (Copenhagen)

Methods	Central telephone randomisation
Participants	Women aged 35 years or over with risk factors including Down syndrome in the family, a previous child with chromosomal abnormality, a parent who was a carrier of chromosomal abnormalities, history of a diseased or dead offspring, recurrent miscarriage, environmental exposure during pregnancy, or anxiety. All women had a singleton pregnancy and gestational age confirmed by ultrasound. Exclusion criteria: high risk of genetic disease (25% or more), malformation suspected on ultrasound, intrauterine device, uterine haematomas, and malformations 579 women were assigned to CVS, 581 women to EA, and 114/1274 (9%) were excluded
Interventions	TA CVS was performed between 10 and 12 weeks with ultrasound guidance and a needle guide. The double needle technique was used (guide needle of 1.2 mm (18 G) and aspiration needle of 0.8 mm (21 G). AC was done between 11 and 13 weeks with a needle guide and a 0.9 mm (20 G) standard AC needle. The filter system was used which allowed circulation of amniotic fluid (25 mL) back to the sac during sampling.
Outcomes	Technical difficulties, abnormal karyotype, pregnancy complications, perinatal loss, neonatal complications.
Notes	Trial was stopped early due to slow recruitment and due to clustering of talipes equinovarus in the EA group. Dates of study: February 1993 - September 1995

Sundberg 1997 (Copenhagen) *(Continued)*

Setting: University Hosiptal, Copenhagen

Funding: not reported

Conflict of interest: not reported

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Method not specified
Allocation concealment (selection bias)	Low risk	Central telephone randomisation
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	All data accounted for
Selective reporting (reporting bias)	Low risk	Specified outcomes reported
Other bias	Low risk	None identified

Tabor 1986

Methods	Random allocation according to a table of random numbers. Randomisation code given out by a medical secretary at Rigshospitalet, Copenhagen (majority). Some women were randomised by envelopes (Fredriksborg county).
Participants	4606 women between ages of 25 and 34 randomised. Exclusion criteria: women believed to be at risk of a child with a chromosomal abnormality, neural tube defect, or increased risk of spontaneous abortion, women with known uterine abnormalities or intrauterine contraceptive devices, multiple gestations
Interventions	Women in the study group were allocated to AC, all of which were carried out at the centre for prenatal diagnosis. The mean gestational age for AC was 16.4 ± 1.1 weeks. AC was carried out with a 20 G needle under direct ultrasound guidance. Women in the control group were allocated to the routine antenatal programme.
Outcomes	Pregnancy outcome, abnormal karyotype and neonatal complications, and congenital abnormalities
Notes	Dates of study: enrolment February 1980 to May 1984 Setting: hospitals in Denmark Funding: This study was supported by the Dagmar Marshall Foundation.

Tabor 1986 *(Continued)*

	Conflict of interest: not reported

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Block randomisation generated using a table of random numbers. Allocated separately at each of 2 recruiting centres.
Allocation concealment (selection bias)	Unclear risk	Methods state 'randomisation numbers kept in sealed opaque envelopes' in 1 centre, no information for second centre.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	3 women lost to follow-up following randomisation were excluded from analysis.
Selective reporting (reporting bias)	Low risk	Specified outcomes reported
Other bias	Low risk	Comparable groups

Tomassini 1988

Methods	Random selection (method unknown)
Participants	44 women between 9 and 12 weeks of gestation
Interventions	Transcervical CVS with ago-cannula, or TA procedure with a spinal needle (G size unknown) and a suction pistol
Outcomes	Sampling failure, vaginal spotting, and amniotic fluid leak, pregnancy loss
Notes	Dates of study: not clear Setting: hospital in Italy Funding: not reported Conflict of interest: not reported

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomisation method not described

Tomassini 1988 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Not feasible due to nature of intervention. Lack of blinding was not expected to have significant impact on the way main outcomes were assessed and recorded (e.g. pregnancy loss, laboratory failure), and therefore, we did not rate the risk of bias as high.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not specified
Incomplete outcome data (attrition bias) All outcomes	Low risk	All participants accounted for
Selective reporting (reporting bias)	Low risk	Specified outcomes reported
Other bias	Low risk	Comparable groups (stated by authors)

AC: amniocentesis
CRL: crown rump length
CVS: chorionic villus sampling
EA: early amniocentesis
G: gauge
IUGR: intrauterine growth restriction
TA: transabdominal
TC: transcervical
vs: versus

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Cederholm 1997 (Uppsala)	The trial randomised 86 women to early amniocentesis or CVS in the Uppsala trial. The data for the 86 randomised women were 'lumped together' with the data for 235 women who selected the procedure 'by choice'. Therefore, at present, we were unable to include the randomised data set in the 'intention-to-treat' analysis.
Chang 1994	This study evaluated the feasibility of midtrimester placental biopsy as an alternative technique of prenatal cytogenetic diagnosis. Mid-trimester amniocentesis and placental biopsies were performed simultaneously in 92 cases. According to our protocol, this type of study design was not included.
Corrado 2002	This study compared a short prophylactic treatment with progesterone after amniocentesis with untreated controls. It did not compare 2 different methods of invasive testing.
Fischer 2000	This study evaluated the role of local anaesthesia in reducing pain during and immediately after the procedure. This study will be included in the Cochrane review that addresses the issue of pain relief during prenatal diagnostic tests.
Gordon 2007	This study evaluated the role of local anaesthesia (1% lidocaine) versus no anaesthesia before amniocentesis in a diverse population. Immediately after the procedure, subjects were asked to assess their pain using both a Visual Analogue Scale and a 101-point Numerical Rating Scale. This

Study	Reason for exclusion
	study will be included in the Cochrane review that addresses the issue of pain relief during prenatal diagnostic tests.
Hewison 2006 (ARIA Trial)	This study evaluated the impact of providing early results in altering maternal anxiety during the waiting period, compared with a policy of telling parents that the results will be issued 'when available' (i.e. variable date). This study will be included in the Cochrane review that addresses the issue of anxiety reduction during prenatal diagnostic tests.
Horovitz 1994	This study compared transabdominal CVS with amniocentesis in 56 multiple pregnancies. It was not clear from the abstract whether this was a randomised study or not.
ISRCTN18010960	This trial assessed amniocentesis for the identification of rapid markers of subclinical chorioamnionitis plus cervical cerclage versus cervical cerclage alone in women with cervical incompetence. The population and indication were outside the scope of this review.
Ketupanya 1997	This study compared early amniocentesis (12 to 14 weeks) performed with or without amniofiltration technique (29 women in each group). The culture failure was 13.8% in the amniofiltration group compared with 10.3% in the control group. However, the method of randomisation was not described.
Leach 1978	In this study, amniocentesis was performed to assess fetal lung maturity with only 10.2% of the procedures carried out before 36 weeks' gestation.
Leung 2002	This study evaluated the impact of early reporting of the results obtained from polymerase chain reaction on amniotic fluid cells (amnio-PCR) on anxiety levels in women with positive biochemical screening for Down syndrome. This study will be included in the Cochrane review that addresses the issue of anxiety reduction during prenatal diagnostic tests.
Levine 1977	This study evaluated the role of ultrasound immediately before genetic amniocentesis. The patients were 'alternately assigned' to the 'with ultrasound' and 'without ultrasound' groups. According to our protocol, quasi-randomised protocols such as alternative allocations were not included.
Pistorius 1998	In this study, amniocentesis was performed later in pregnancy in women with proteinuric hypertension.
Shalev 1994	This is an abstract of the study that compared the clinical and laboratory result of first trimester transvaginal amniocentesis with those of CVS and mid-trimester amniocentesis. It had a matched case-control study design. It did not meet inclusion criteria of this review.
Shulman 1990	This study reported on a comparison between 15 transcervical and 15 transabdominal CVS procedures in terms of the specimen size and change in maternal serum alpha-feto-protein levels. Some women were selected by 'choice' and others took part in the NICH study comparing CVS and amniocentesis (Rhoads GG, Jackson LG, Schlesselman SE, de la Cruz FF, Desnick RJ, Golbus MS et al. The safety and efficacy of CVS for early prenatal diagnosis of cytogenetic abnormalities. New England Journal of Medicine 1989;320(10):609-17). Therefore, this study did not fulfil our criteria for randomised study.
SIlver 2005	This study was a part of a randomised control study performed by NICHD EATA Trial Group. It evaluated the relationship between placental penetration during amniocentesis or CVS and the development of gestational hypertension/pre-eclampsia.
Van Schoubroeck 2000	This study evaluated the role of therapeutic massage in reducing pain during and immediately after the procedure. This study will be included in the Cochrane review that addresses the issue of pain relief during prenatal diagnostic tests.

Study	Reason for exclusion
Wax 2005	This study determined whether pain associated with second trimester genetic amniocentesis was decreased by using subfreezing rather than room temperature needles. This study will be included in the Cochrane review that addresses the issue of pain relief during prenatal diagnostic tests.
Zwinger 1994	This study evaluated the efficiency and safety of individual invasive methods of prenatal diagnosis. This study was not a randomised controlled study but was based on a population cohort of Institute for Mother and Child Care in Czech Republic. Data were represented in an abstract form for the conference proceeding.

CVS: chorionic villus sampling

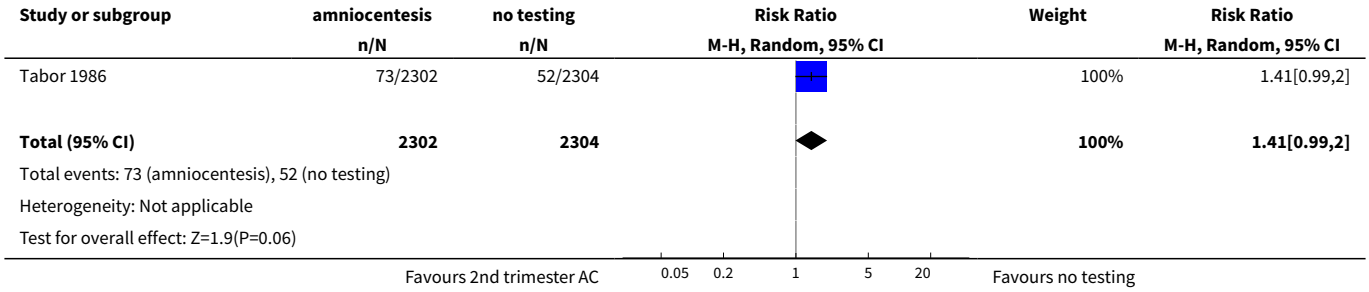
DATA AND ANALYSES

Comparison 1. Second trimester amniocentesis (AC) versus control (no testing)

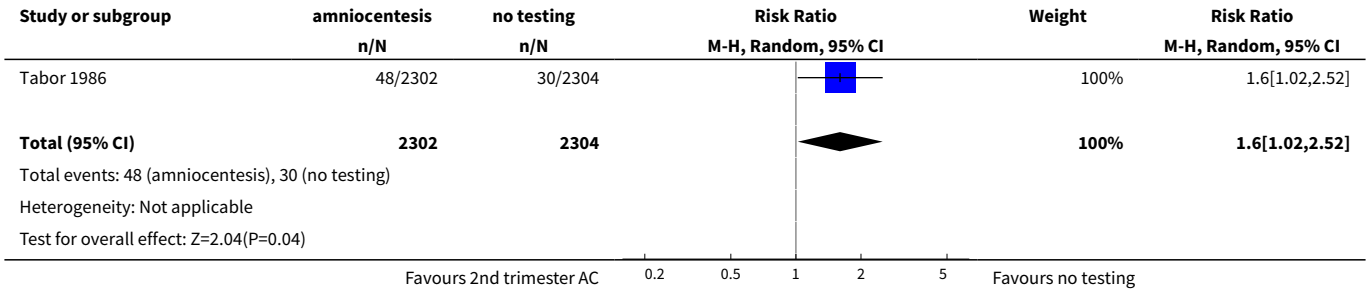
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All known pregnancy loss (including termination of pregnancy)	1	4606	Risk Ratio (M-H, Random, 95% CI)	1.41 [0.99, 2.00]
2 Spontaneous miscarriage	1	4606	Risk Ratio (M-H, Random, 95% CI)	1.60 [1.02, 2.52]
3 Non-compliance with allocated procedure	1	4606	Risk Ratio (M-H, Random, 95% CI)	1.73 [1.03, 2.91]
4 Multiple insertions	1	4606	Risk Ratio (M-H, Random, 95% CI)	91.08 [5.61, 1477.53]
5 Second test performed	1	4606	Risk Ratio (M-H, Random, 95% CI)	41.04 [2.48, 678.07]
6 Laboratory failure	1	4606	Risk Ratio (M-H, Random, 95% CI)	27.02 [1.61, 454.31]
7 All non-mosaic abnormalities	1	4593	Risk Ratio (M-H, Random, 95% CI)	30.85 [1.85, 515.31]
8 Vaginal bleeding after test	1	4606	Risk Ratio (M-H, Random, 95% CI)	0.95 [0.66, 1.37]
9 Amniotic leakage after test	1	4606	Risk Ratio (M-H, Random, 95% CI)	3.90 [1.95, 7.80]
10 Termination of pregnancy (all)	1	4606	Risk Ratio (M-H, Random, 95% CI)	2.50 [0.97, 6.44]
11 Perinatal deaths	1	4606	Risk Ratio (M-H, Random, 95% CI)	0.63 [0.28, 1.38]
12 Stillbirths	1	4606	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.36, 1.93]
13 Neonatal deaths	1	4606	Risk Ratio (M-H, Random, 95% CI)	0.11 [0.01, 2.06]
14 All recorded deaths after viability	1	4606	Risk Ratio (M-H, Random, 95% CI)	0.63 [0.28, 1.38]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
15 Anomalies (all recorded)	1	4507	Risk Ratio (M-H, Random, 95% CI)	0.93 [0.62, 1.39]
16 Talipes	1	4507	Risk Ratio (M-H, Random, 95% CI)	0.68 [0.37, 1.22]
17 Neonatal respiratory distress syndrome	1	4507	Risk Ratio (M-H, Random, 95% CI)	2.11 [1.06, 4.19]

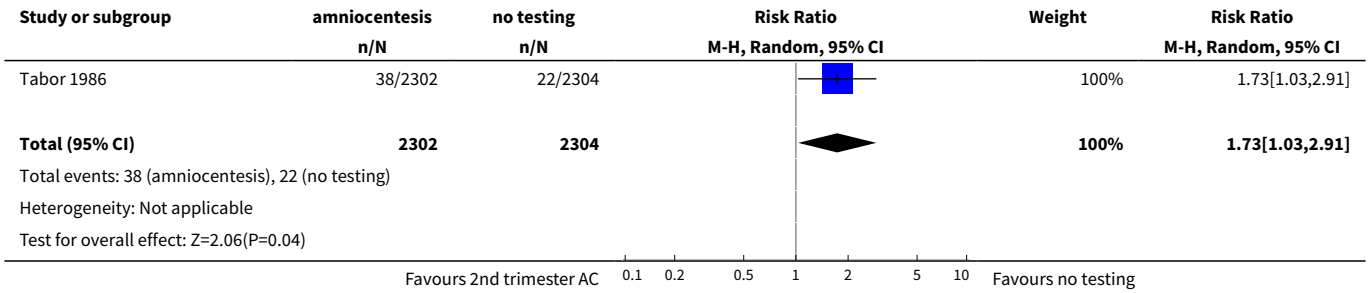
Analysis 1.1. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 1 All known pregnancy loss (including termination of pregnancy).



Analysis 1.2. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 2 Spontaneous miscarriage.

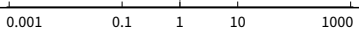


Analysis 1.3. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 3 Non-compliance with allocated procedure.



Analysis 1.4. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 4 Multiple insertions.

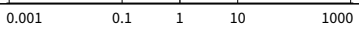
Study or subgroup	amniocentesis n/N	no testing n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Tabor 1986	45/2302	0/2304		100%	91.08[5.61,1477.53]
Total (95% CI)	**2302**	**2304**		**100%**	**91.08[5.61,1477.53]**
Total events: 45 (amniocentesis), 0 (no testing)					
Heterogeneity: Not applicable					
Test for overall effect: Z=3.17(P=0)					



Favours 2nd trimester AC | Favours no testing

Analysis 1.5. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 5 Second test performed.

Study or subgroup	amniocentesis n/N	no testing n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Tabor 1986	20/2302	0/2304		100%	41.04[2.48,678.07]
Total (95% CI)	**2302**	**2304**		**100%**	**41.04[2.48,678.07]**
Total events: 20 (amniocentesis), 0 (no testing)					
Heterogeneity: Not applicable					
Test for overall effect: Z=2.6(P=0.01)					



Favours 2nd trimester AC | Favours no testing

Analysis 1.6. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 6 Laboratory failure.

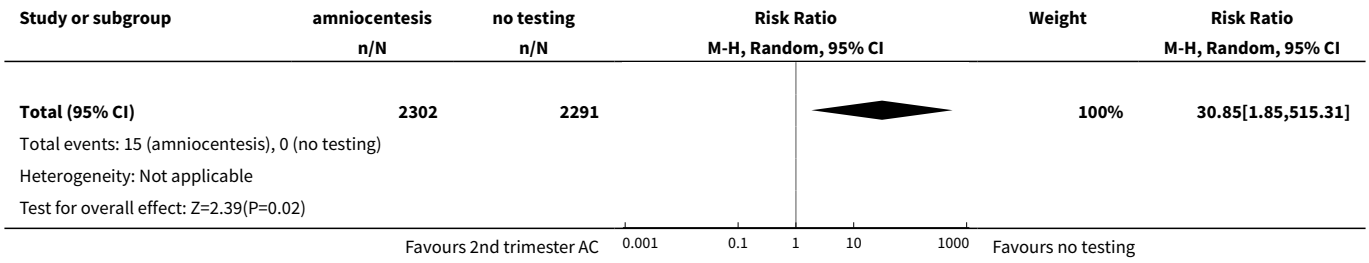
Study or subgroup	amniocentesis n/N	no testing n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Tabor 1986	13/2302	0/2304		100%	27.02[1.61,454.31]
Total (95% CI)	**2302**	**2304**		**100%**	**27.02[1.61,454.31]**
Total events: 13 (amniocentesis), 0 (no testing)					
Heterogeneity: Not applicable					
Test for overall effect: Z=2.29(P=0.02)					

Favours 2nd trimester AC | Favours no testing

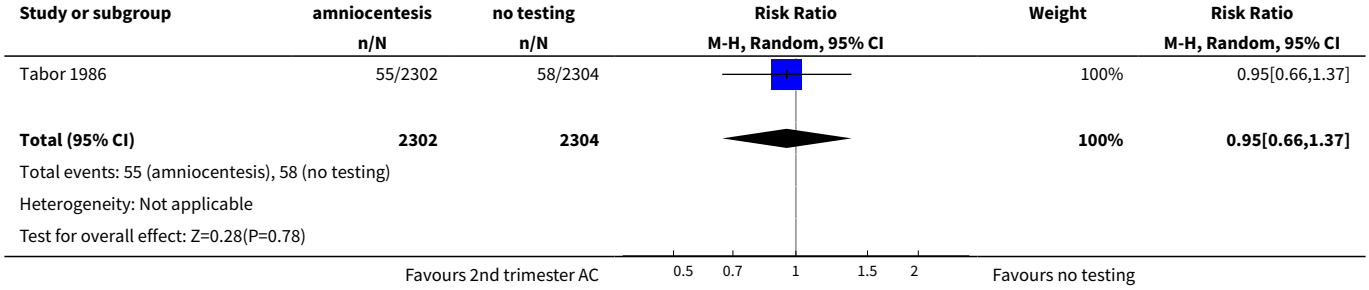
Analysis 1.7. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 7 All non-mosaic abnormalities.

Study or subgroup	amniocentesis n/N	no testing n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Tabor 1986	15/2302	0/2291		100%	30.85[1.85,515.31]

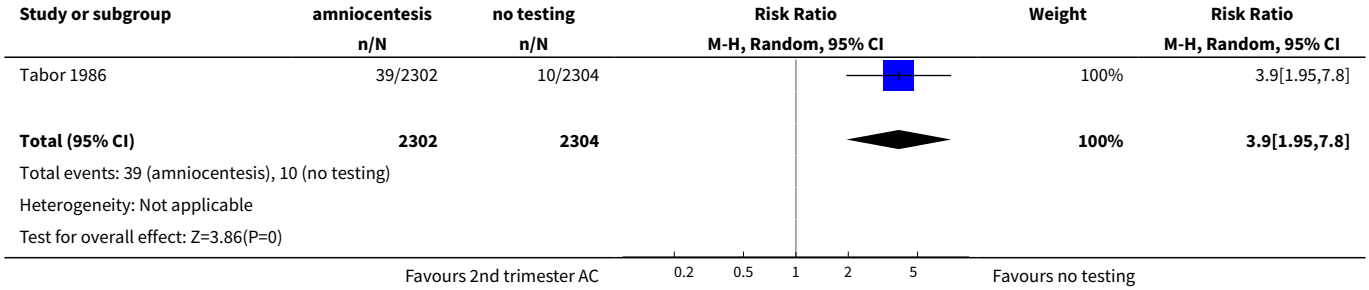
Favours 2nd trimester AC | Favours no testing



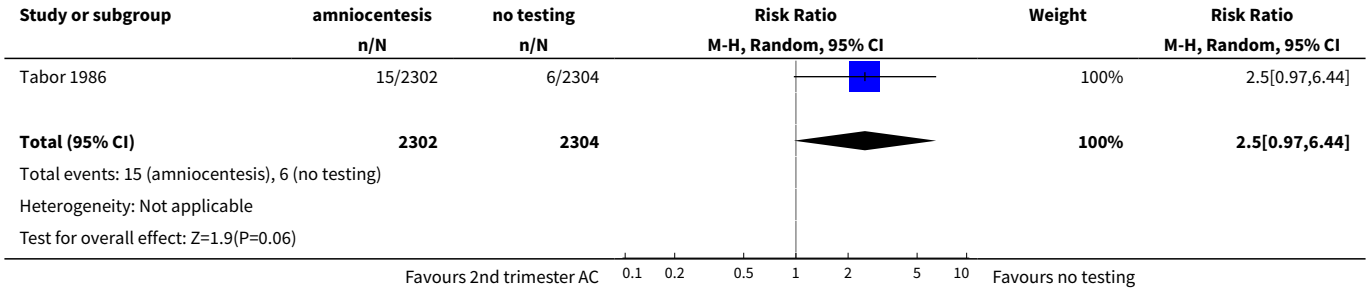
Analysis 1.8. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 8 Vaginal bleeding after test.



Analysis 1.9. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 9 Amniotic leakage after test.

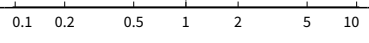


Analysis 1.10. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 10 Termination of pregnancy (all).



Analysis 1.11. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 11 Perinatal deaths.

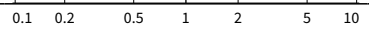
Study or subgroup	amniocentesis n/N	no testing n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Tabor 1986	10/2302	16/2304		100%	0.63[0.28,1.38]
Total (95% CI)	**2302**	**2304**		**100%**	**0.63[0.28,1.38]**
Total events: 10 (amniocentesis), 16 (no testing)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.17(P=0.24)					



Favours 2nd trimester AC — Favours no testing

Analysis 1.12. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 12 Stillbirths.

Study or subgroup	amniocentesis n/N	no testing n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Tabor 1986	10/2302	12/2304		100%	0.83[0.36,1.93]
Total (95% CI)	**2302**	**2304**		**100%**	**0.83[0.36,1.93]**
Total events: 10 (amniocentesis), 12 (no testing)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.42(P=0.67)					



Favours 2nd trimester AC — Favours no testing

Analysis 1.13. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 13 Neonatal deaths.

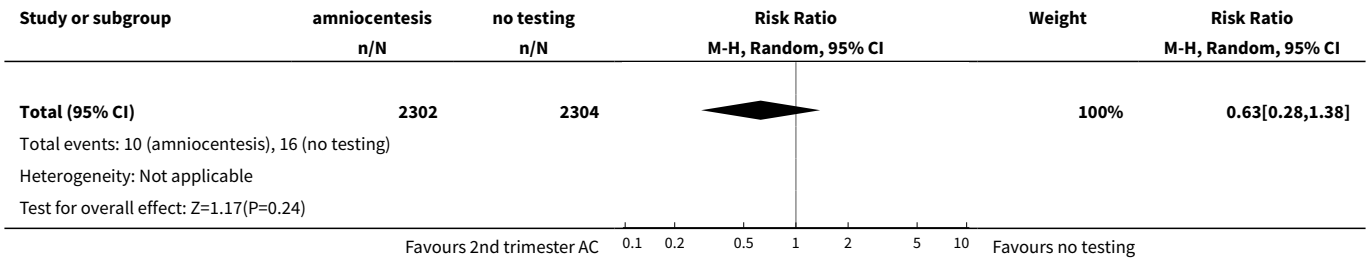
Study or subgroup	amniocentesis n/N	no testing n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Tabor 1986	0/2302	4/2304		100%	0.11[0.01,2.06]
Total (95% CI)	**2302**	**2304**		**100%**	**0.11[0.01,2.06]**
Total events: 0 (amniocentesis), 4 (no testing)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.47(P=0.14)					

Favours 2nd trimester AC — Favours no testing

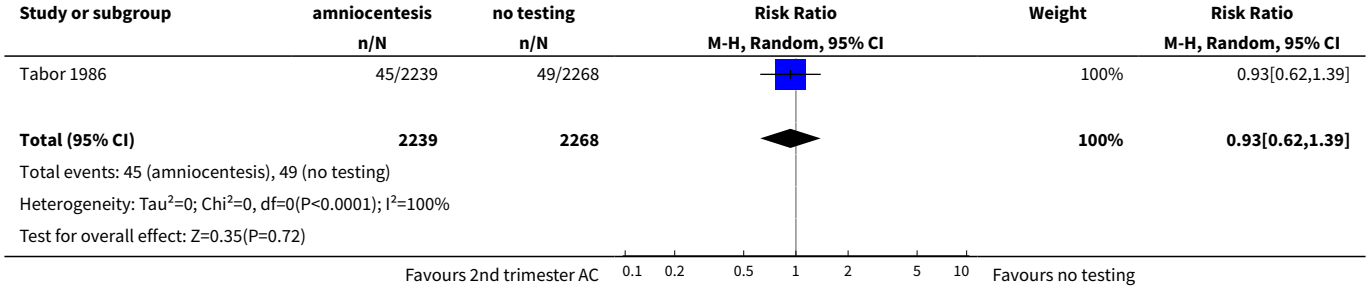
Analysis 1.14. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 14 All recorded deaths after viability.

Study or subgroup	amniocentesis n/N	no testing n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Tabor 1986	10/2302	16/2304		100%	0.63[0.28,1.38]

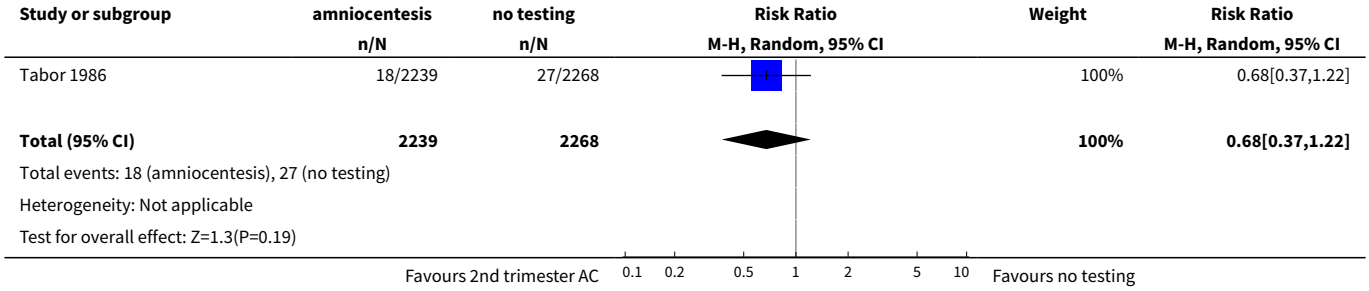
Favours 2nd trimester AC — Favours no testing



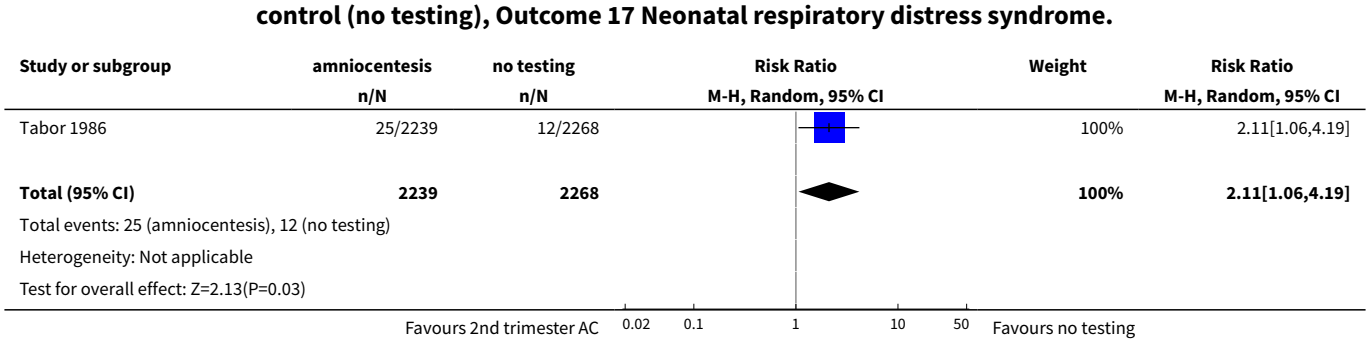
Analysis 1.15. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 15 Anomalies (all recorded).



Analysis 1.16. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 16 Talipes.



Analysis 1.17. Comparison 1 Second trimester amniocentesis (AC) versus control (no testing), Outcome 17 Neonatal respiratory distress syndrome.

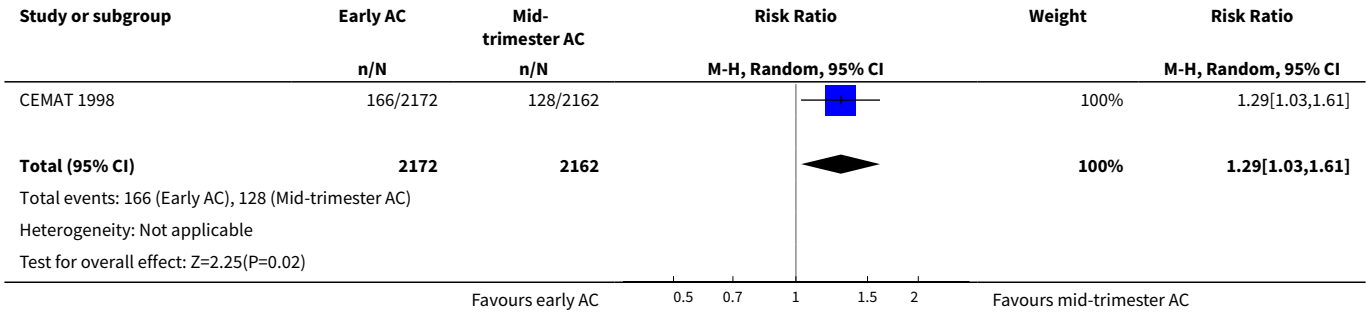


Comparison 2. Early versus second trimester amniocentesis (AC)

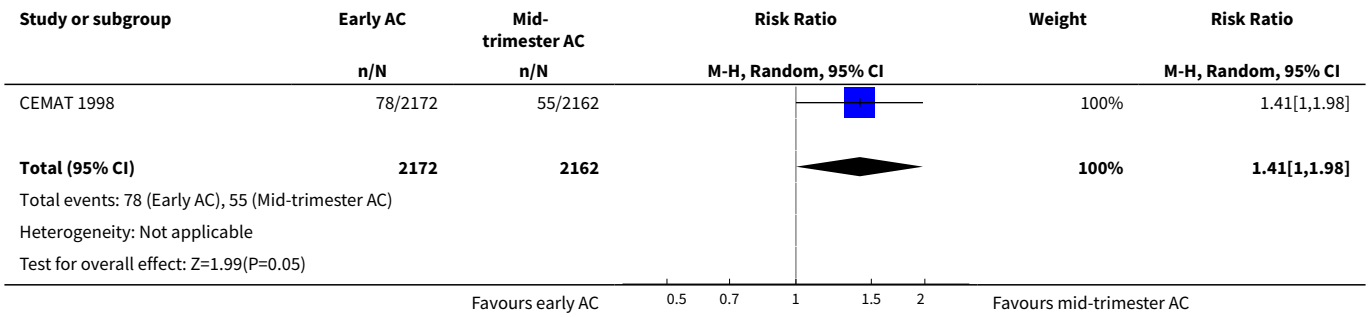
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All known pregnancy loss (including termination of pregnancy)	1	4334	Risk Ratio (M-H, Random, 95% CI)	1.29 [1.03, 1.61]
2 Spontaneous miscarriage	1	4334	Risk Ratio (M-H, Random, 95% CI)	1.41 [1.00, 1.98]
3 Spontaneous miscarriage after test	1	4334	Risk Ratio (M-H, Random, 95% CI)	3.22 [1.88, 5.53]
4 Non-compliance with allocated procedure	1	4368	Risk Ratio (M-H, Random, 95% CI)	0.65 [0.57, 0.75]
5 Sampling failure	1	629	Risk Ratio (M-H, Random, 95% CI)	4.53 [0.53, 38.56]
6 Multiple insertions	1	4368	Risk Ratio (M-H, Random, 95% CI)	2.79 [1.92, 4.04]
7 Second test performed	1	4107	Risk Ratio (M-H, Random, 95% CI)	8.72 [3.47, 21.91]
8 Laboratory failure	1	4368	Risk Ratio (M-H, Random, 95% CI)	9.76 [3.49, 27.26]
9 All non-mosaic abnormalities	1	4368	Risk Ratio (M-H, Random, 95% CI)	1.11 [0.75, 1.66]
10 True mosaics	1	4368	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.25, 4.00]
11 Maternal contamination	1	4368	Risk Ratio (M-H, Random, 95% CI)	2.00 [0.37, 10.92]
12 Known false negative after birth	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
12.1 False negative chromosomal results (excluding sex determination)	1	4368	Risk Ratio (M-H, Random, 95% CI)	3.00 [0.12, 73.67]
12.2 Incorrect sex determination	1	4368	Risk Ratio (M-H, Random, 95% CI)	5.00 [0.24, 104.18]
13 Reporting time	1	4107	Mean Difference (IV, Random, 95% CI)	1.20 [0.89, 1.51]
14 Amniotic leakage after test	1	4368	Risk Ratio (M-H, Random, 95% CI)	2.05 [1.43, 2.94]
15 Termination of pregnancy (all)	1	4334	Risk Ratio (M-H, Random, 95% CI)	1.26 [0.89, 1.77]
16 Stillbirths	1	4334	Risk Ratio (M-H, Random, 95% CI)	0.73 [0.34, 1.59]
17 Neonatal deaths	1	4334	Risk Ratio (M-H, Random, 95% CI)	4.98 [0.58, 42.56]
18 All recorded deaths after viability	1	4334	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.50, 1.99]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
19 Anomalies (all recorded)	1	4334	Risk Ratio (M-H, Random, 95% CI)	1.73 [1.26, 2.38]
20 Talipes	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
20.1 Talipes equinovarus	1	4334	Risk Ratio (M-H, Random, 95% CI)	14.43 [3.45, 60.41]

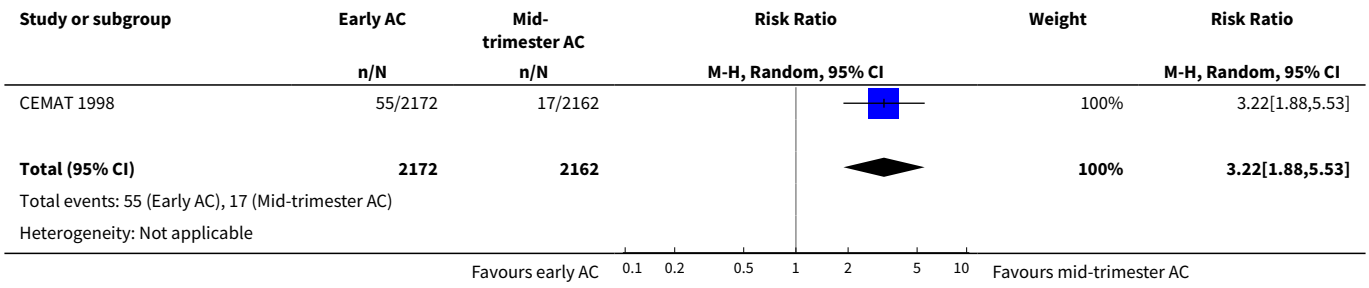
Analysis 2.1. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 1 All known pregnancy loss (including termination of pregnancy).

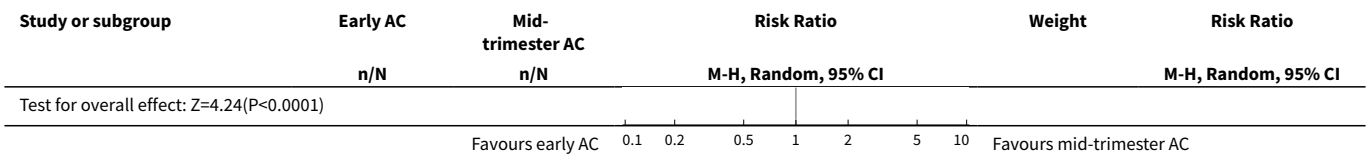


Analysis 2.2. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 2 Spontaneous miscarriage.

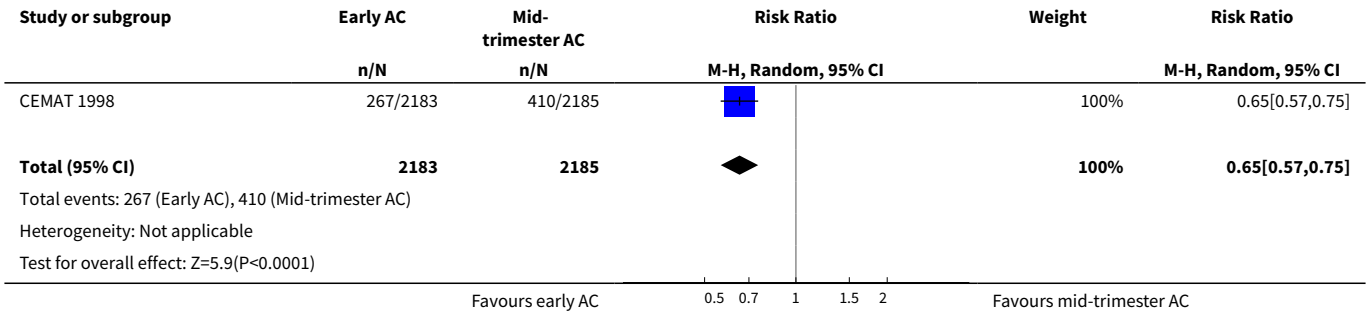


Analysis 2.3. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 3 Spontaneous miscarriage after test.

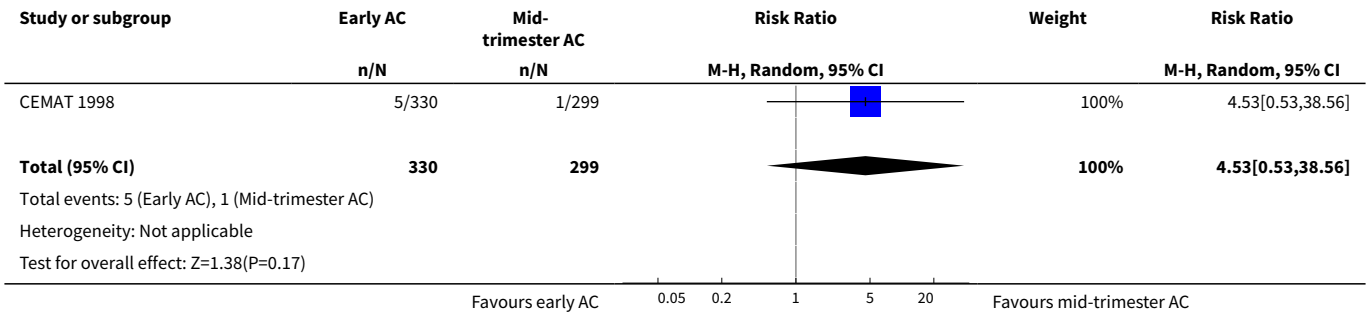




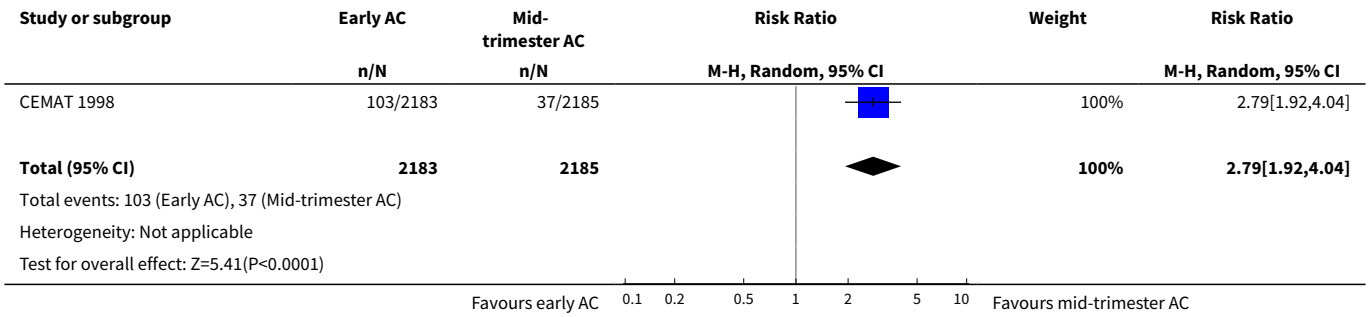
Analysis 2.4. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 4 Non-compliance with allocated procedure.



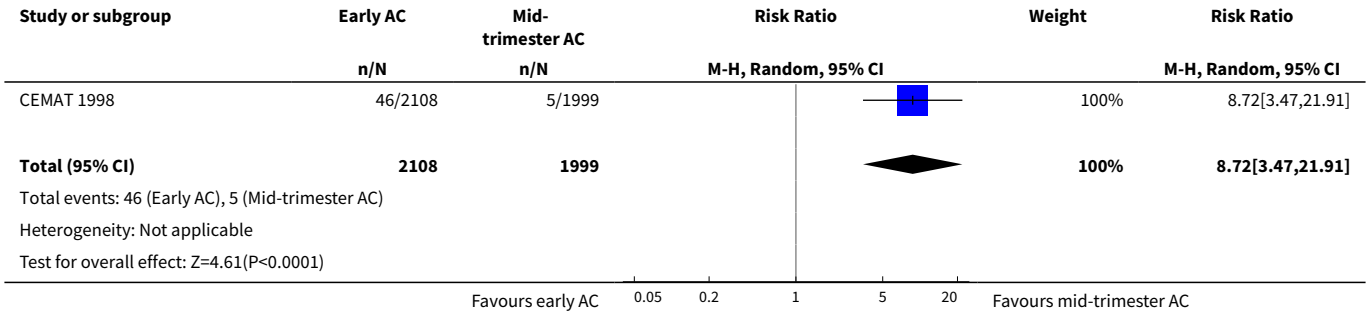
Analysis 2.5. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 5 Sampling failure.



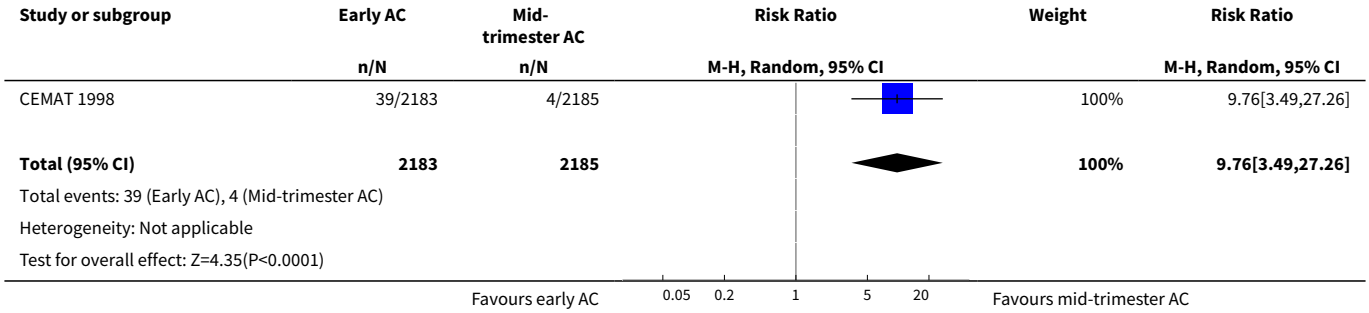
Analysis 2.6. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 6 Multiple insertions.



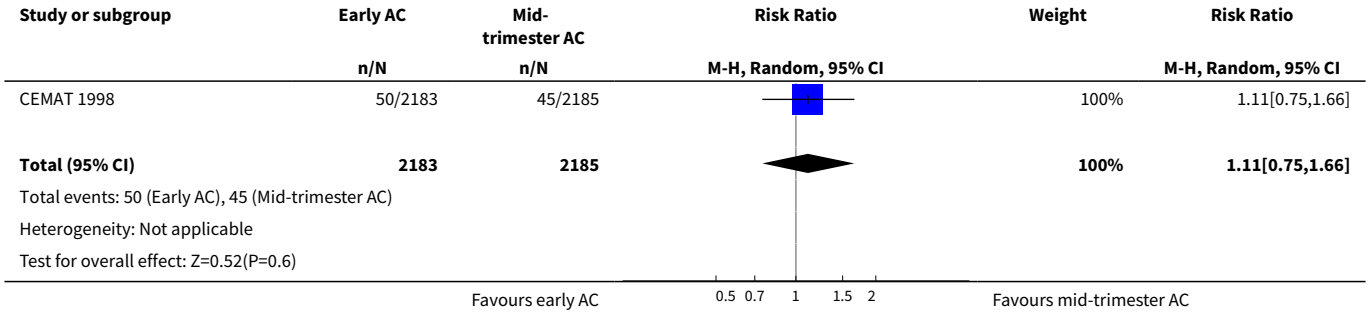
Analysis 2.7. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 7 Second test performed.



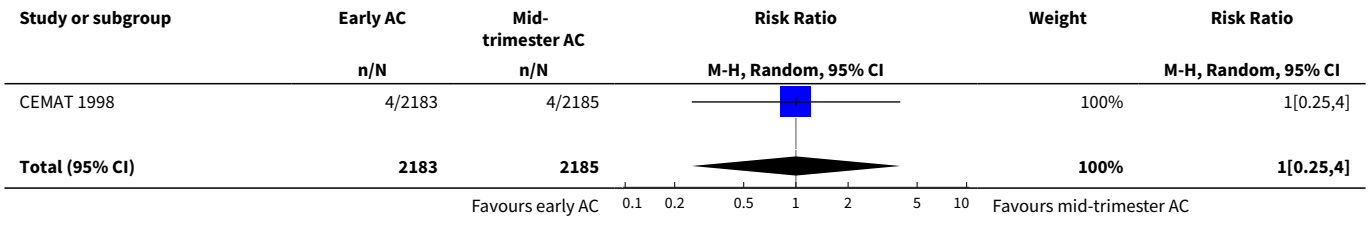
Analysis 2.8. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 8 Laboratory failure.

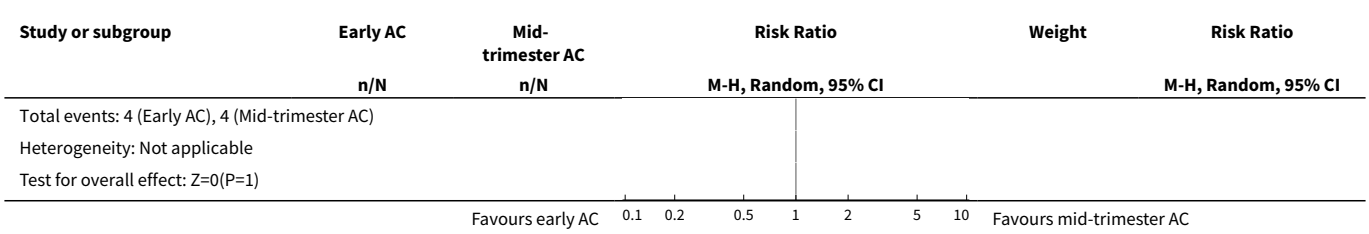


Analysis 2.9. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 9 All non-mosaic abnormalities.

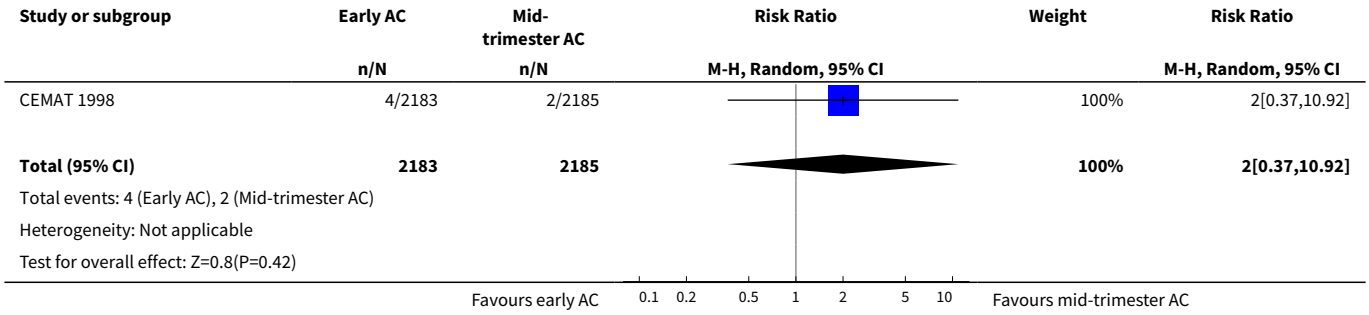


Analysis 2.10. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 10 True mosaics.

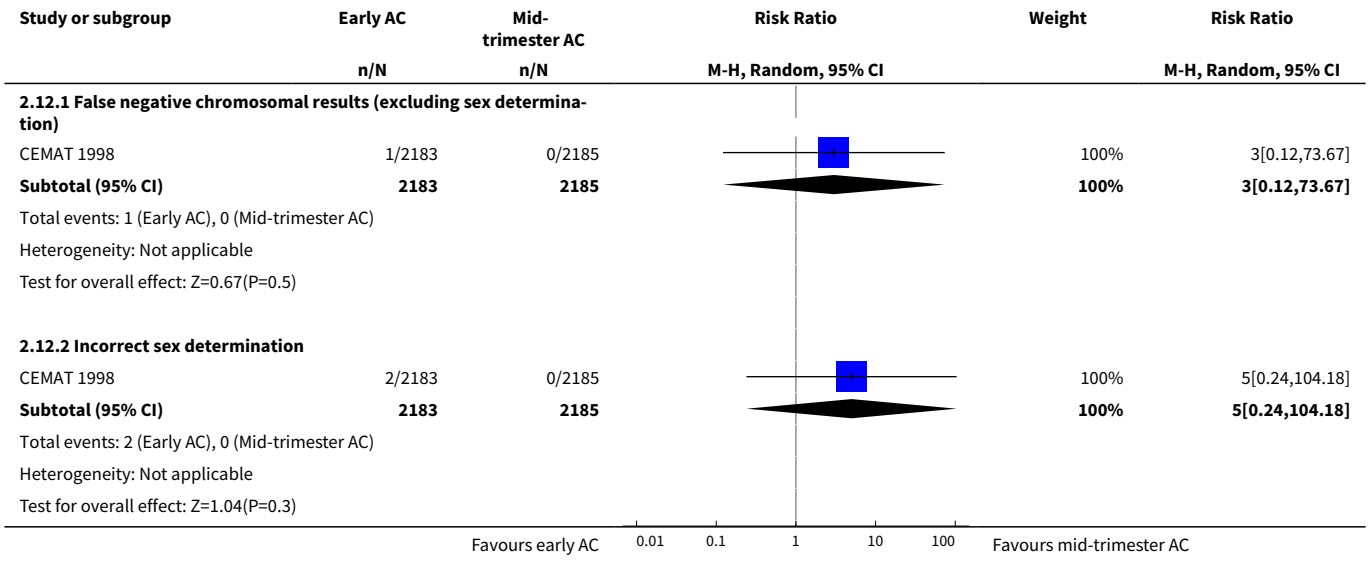




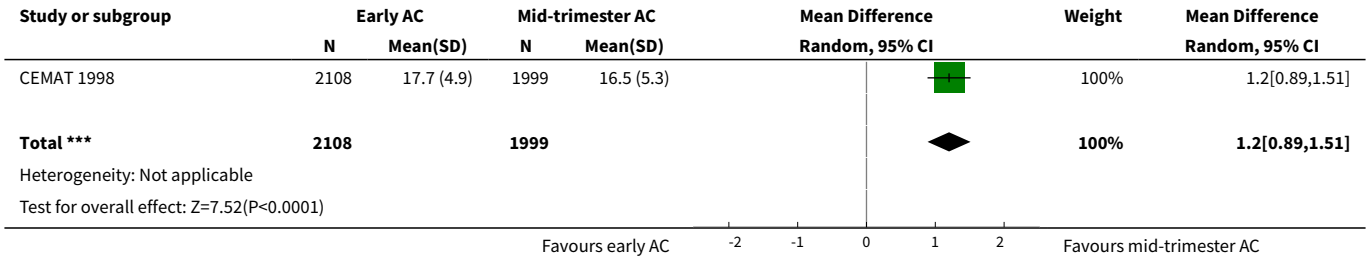
Analysis 2.11. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 11 Maternal contamination.



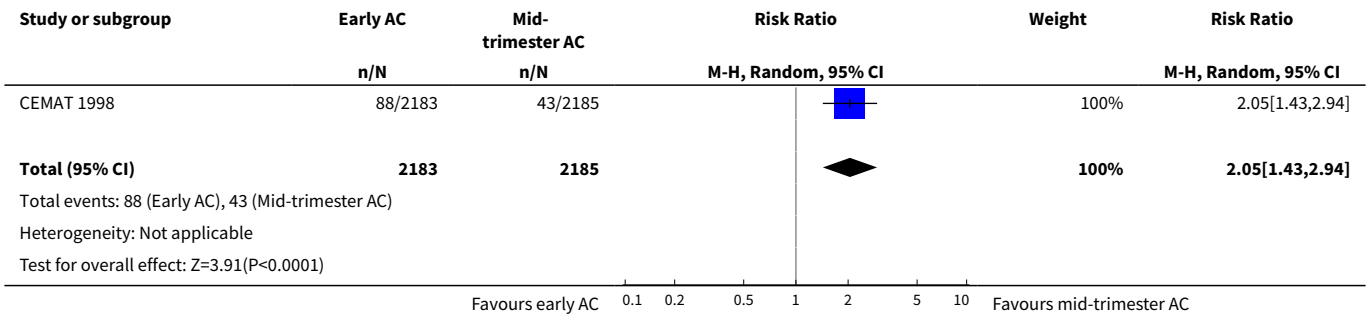
Analysis 2.12. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 12 Known false negative after birth.



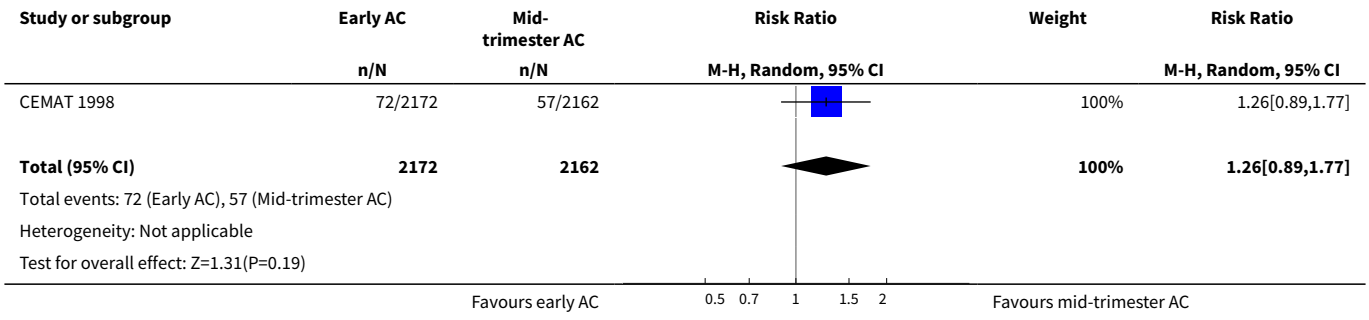
Analysis 2.13. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 13 Reporting time.



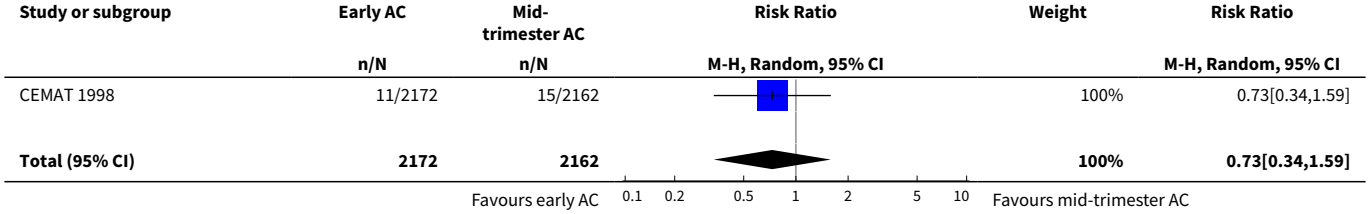
Analysis 2.14. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 14 Amniotic leakage after test.

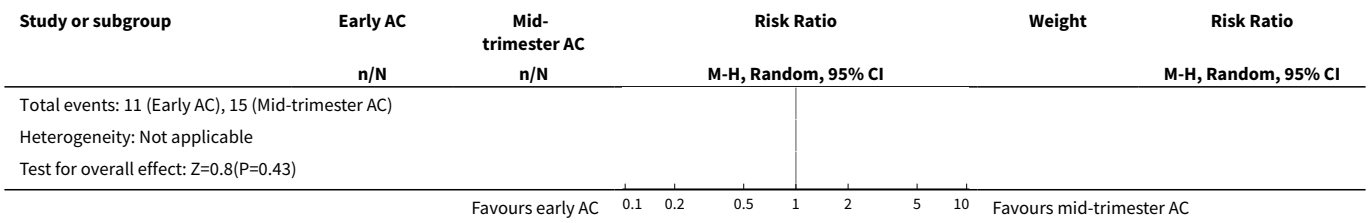


Analysis 2.15. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 15 Termination of pregnancy (all).

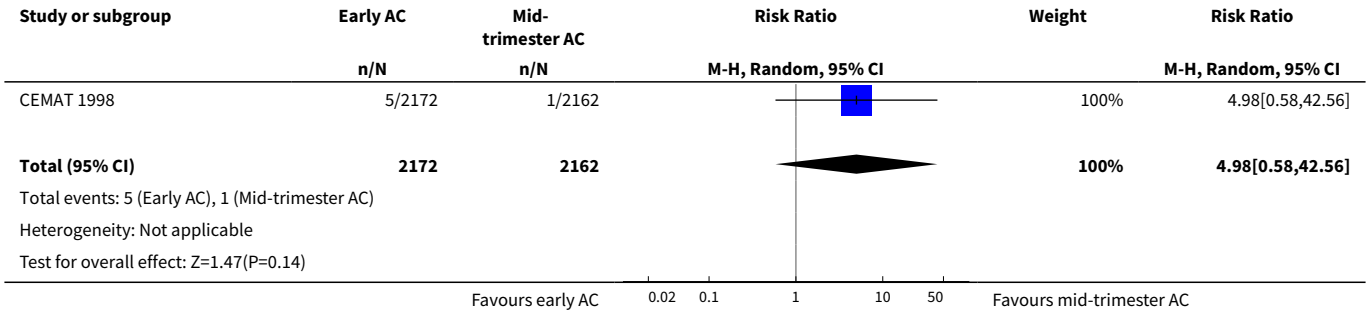


Analysis 2.16. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 16 Stillbirths.

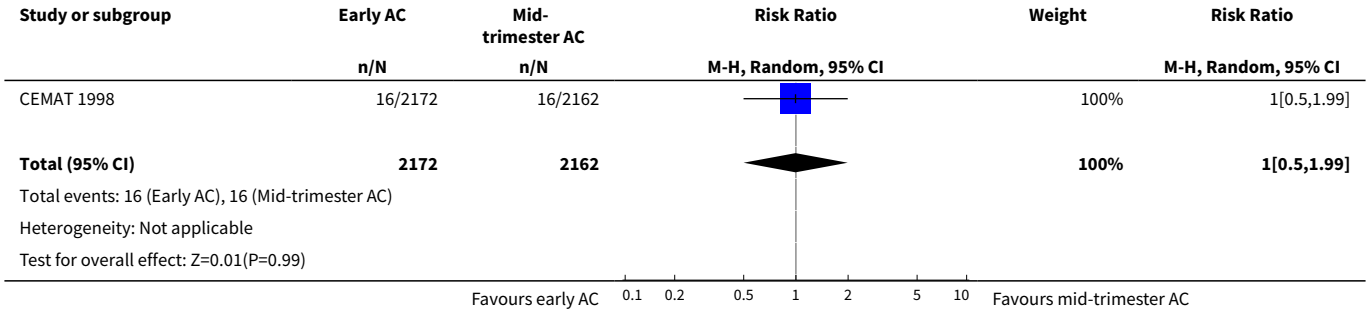




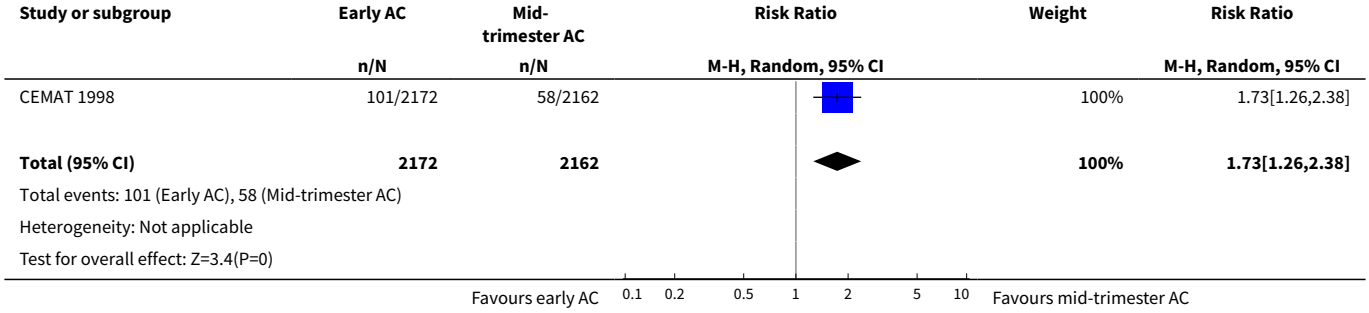
Analysis 2.17. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 17 Neonatal deaths.



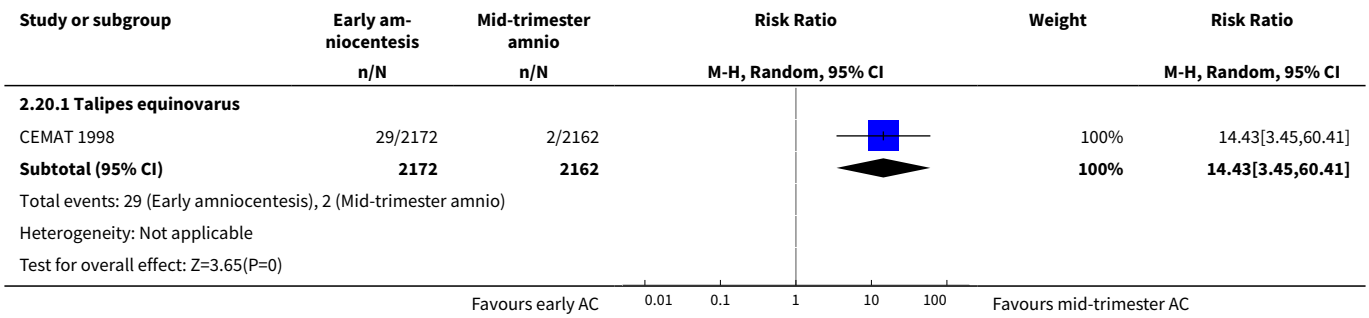
Analysis 2.18. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 18 All recorded deaths after viability.



Analysis 2.19. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 19 Anomalies (all recorded).



Analysis 2.20. Comparison 2 Early versus second trimester amniocentesis (AC), Outcome 20 Talipes.



Comparison 3. Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All known pregnancy loss (including termination of pregnancy)	5		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.1 Transcervical CVS versus amniocentesis	4	6527	Risk Ratio (M-H, Random, 95% CI)	1.40 [1.09, 1.81]
1.2 Transabdominal CVS versus amniocentesis	1	2234	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.66, 1.23]
1.3 CVS (any route) versus amniocentesis	2	6503	Risk Ratio (M-H, Random, 95% CI)	1.43 [1.22, 1.67]
2 Spontaneous miscarriage	4		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.1 Transcervical CVS versus amniocentesis	3	5506	Risk Ratio (M-H, Random, 95% CI)	1.50 [1.07, 2.11]
2.2 Transabdominal CVS versus amniocentesis	1	2069	Risk Ratio (M-H, Random, 95% CI)	0.77 [0.49, 1.21]
2.3 CVS (any route) versus amniocentesis	2	6280	Risk Ratio (M-H, Random, 95% CI)	1.51 [1.23, 1.85]
3 Spontaneous miscarriage after test	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
3.1 Transcervical CVS versus amniocentesis	2	1579	Risk Ratio (M-H, Random, 95% CI)	1.77 [0.28, 11.00]
3.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	3.46 [2.21, 5.42]
4 Non-compliance with allocated procedure	4		Risk Ratio (M-H, Random, 95% CI)	Subtotals only

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 Transcervical CVS versus amniocentesis	3	4595	Risk Ratio (M-H, Random, 95% CI)	0.51 [0.14, 1.87]
4.2 CVS (any route) versus amniocentesis	1	3197	Risk Ratio (M-H, Random, 95% CI)	0.66 [0.52, 0.83]
5 Sampling failure	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
5.1 Transervical CVS versus amniocentesis	1	797	Risk Ratio (M-H, Random, 95% CI)	0.55 [0.26, 1.19]
5.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	3.09 [1.98, 4.82]
6 Multiple insertions	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
6.1 Transcervical CVS versus amniocentesis	1	794	Risk Ratio (M-H, Random, 95% CI)	3.93 [2.72, 5.68]
6.2 CVS (any route) versus amniocentesis	1	2917	Risk Ratio (M-H, Random, 95% CI)	4.85 [3.92, 6.01]
7 Second test performed	4		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
7.1 Transcervical CVS versus amniocentesis	3	4256	Risk Ratio (M-H, Random, 95% CI)	19.63 [1.24, 309.90]
7.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	2.83 [1.94, 4.13]
8 Laboratory failure	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
8.1 Transcervical CVS versus amniocentesis	2	2792	Risk Ratio (M-H, Random, 95% CI)	22.62 [3.07, 166.89]
8.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	0.77 [0.29, 2.06]
9 All non-mosaic abnormalities	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
9.1 Transcervical CVS versus amniocentesis	2	2667	Risk Ratio (M-H, Random, 95% CI)	1.12 [0.73, 1.72]
10 True mosaics	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
10.1 Transcervical CVS versus amniocentesis	1	672	Risk Ratio (M-H, Random, 95% CI)	3.42 [0.14, 83.63]

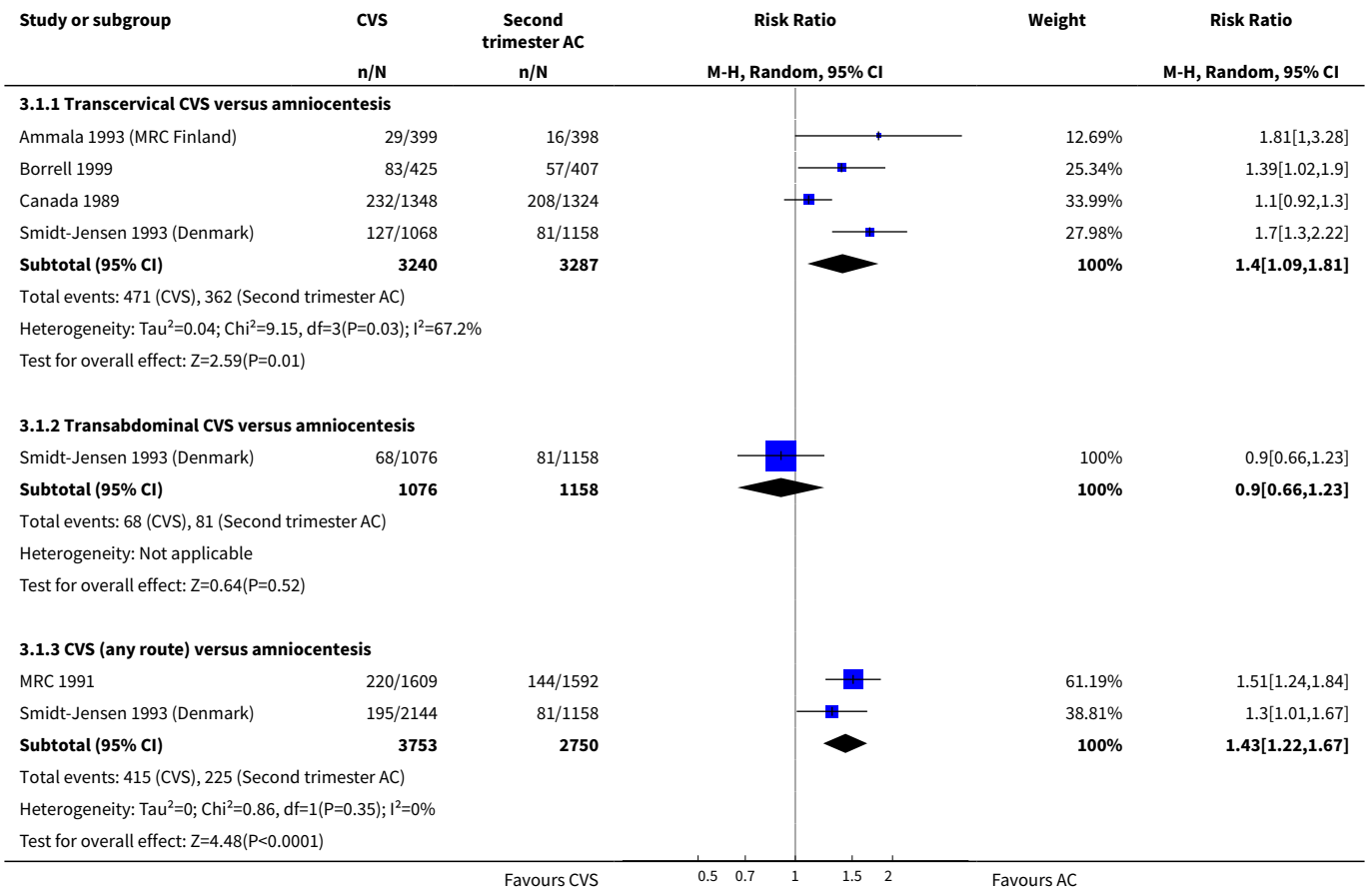
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
11 Confined mosaics	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
11.1 Transcervical CVS versus amniocentesis	1	1995	Risk Ratio (M-H, Random, 95% CI)	5.66 [1.97, 16.24]
12 Maternal contamination	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
12.1 Transcervical CVS versus amniocentesis	1	1991	Risk Ratio (M-H, Random, 95% CI)	12.30 [3.81, 39.67]
12.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	8.90 [0.48, 165.26]
13 Known false positive after birth	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
13.1 Transcervical CVS versus amniocentesis	2	2627	Risk Ratio (M-H, Random, 95% CI)	4.40 [0.46, 42.38]
13.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	0.99 [0.06, 15.80]
14 Known false negative after birth	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
14.1 Transcervical CVS versus amniocentesis	2	2627	Risk Ratio (M-H, Random, 95% CI)	7.84 [0.41, 151.61]
14.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	2.97 [0.12, 72.81]
15 Results given in less than 7 days (not pre-specified)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
15.1 CVS (any route) versus amniocentesis	1	3099	Risk Ratio (M-H, Random, 95% CI)	23.52 [12.54, 44.10]
16 Results given in less than 14 days (not pre-specified)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
16.1 CVS (any route) versus amniocentesis	1	3099	Risk Ratio (M-H, Random, 95% CI)	3.96 [3.17, 4.95]
17 Results given in less than 21 days (not pre-specified)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
17.1 CVS (any route) versus amniocentesis	1	3099	Risk Ratio (M-H, Random, 95% CI)	0.72 [0.63, 0.82]
18 Result given after 21 days (not pre-specified)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
18.1 CVS (any route) versus amniocentesis	1	3099	Risk Ratio (M-H, Random, 95% CI)	0.33 [0.28, 0.39]
19 Vaginal bleeding after test	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
19.1 Transcervical CVS versus amniocentesis	2	3193	Risk Ratio (M-H, Random, 95% CI)	11.48 [2.58, 51.08]
20 Amniotic leakage after test	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
20.1 Transabdominal CVS vs amniocentesis	1	1485	Risk Ratio (M-H, Random, 95% CI)	2.53 [0.81, 7.92]
20.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	0.55 [0.18, 1.64]
21 Vaginal bleeding after 20 weeks	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
21.1 Transcervical CVS versus amniocentesis	1	797	Risk Ratio (M-H, Random, 95% CI)	1.44 [0.62, 3.33]
21.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	0.99 [0.69, 1.42]
22 Pre-labour ruptured membranes before 28 weeks	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
22.1 Transcervical CVS versus amniocentesis	1	722	Risk Ratio (M-H, Random, 95% CI)	4.97 [1.45, 17.03]
22.2 CVS (any route) versus amniocentesis	1	2765	Risk Ratio (M-H, Random, 95% CI)	1.60 [0.80, 3.17]
23 Antenatal hospital admission	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
23.1 Transcervical CVS versus amniocentesis	1	780	Risk Ratio (M-H, Random, 95% CI)	1.47 [0.81, 2.68]
23.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.75, 1.08]
24 Delivery before 37 weeks	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
24.1 Transcervical CVS versus amniocentesis	2	2506	Risk Ratio (M-H, Random, 95% CI)	1.29 [0.67, 2.47]
24.2 CVS (any route) versus amniocentesis	1	3189	Risk Ratio (M-H, Random, 95% CI)	1.33 [1.13, 1.57]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
25 Delivery before 33 weeks	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
25.1 Transcervical CVS versus amniocentesis	1	768	Risk Ratio (M-H, Random, 95% CI)	2.16 [0.94, 4.94]
26 Termination of pregnancy (all)	3		Risk Difference (M-H, Random, 95% CI)	Subtotals only
26.1 Transcervical CVS versus amniocentesis	2	3454	Risk Difference (M-H, Random, 95% CI)	-0.00 [-0.01, 0.01]
26.2 CVS (any route) versus amniocentesis	1	3201	Risk Difference (M-H, Random, 95% CI)	0.01 [-0.00, 0.02]
27 Perinatal deaths	4		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
27.1 Transcervical CVS versus amniocentesis	3	5521	Risk Ratio (M-H, Random, 95% CI)	1.79 [0.42, 7.69]
27.2 Transabdominal CVS versus amniocentesis	1	2069	Risk Ratio (M-H, Random, 95% CI)	1.18 [0.40, 3.51]
27.3 CVS (any route) versus amniocentesis	2	6280	Risk Ratio (M-H, Random, 95% CI)	1.20 [0.64, 2.24]
28 Stillbirths	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
28.1 Transcervical CVS versus amniocentesis	2	3454	Risk Ratio (M-H, Random, 95% CI)	0.94 [0.02, 45.31]
28.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	0.99 [0.35, 2.81]
29 Neonatal deaths	4		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
29.1 Transcervical CVS versus amniocentesis	3	4251	Risk Ratio (M-H, Random, 95% CI)	1.63 [0.38, 7.05]
29.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	2.64 [0.70, 9.93]
30 All recorded deaths after viability	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
30.1 Transcervical CVS versus amniocentesis	2	1579	Risk Ratio (M-H, Random, 95% CI)	0.78 [0.02, 25.93]
30.2 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	1.44 [0.67, 3.09]

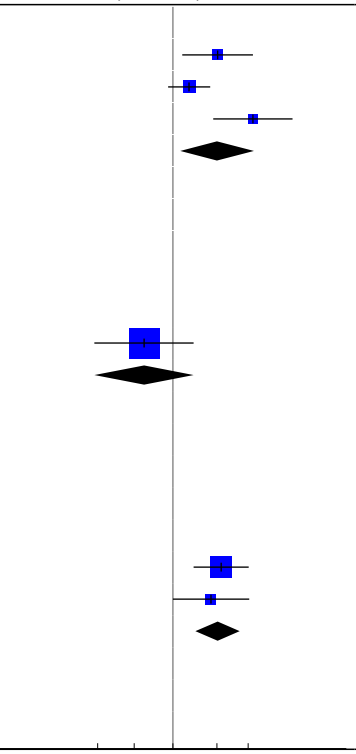
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
31 Congenital anomalies (all recorded)	4		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
31.1 Transcervical CVS versus amniocentesis	2	1408	Risk Ratio (M-H, Random, 95% CI)	0.62 [0.25, 1.59]
31.2 CVS (any route) versus amniocentesis	2	3338	Risk Ratio (M-H, Random, 95% CI)	0.77 [0.66, 0.89]
32 Haemangioma	1	182	Risk Ratio (M-H, Random, 95% CI)	1.35 [0.81, 2.24]
33 Limb reduction defects	1	3201	Risk Ratio (M-H, Random, 95% CI)	4.95 [0.24, 102.97]
33.1 CVS (any route) versus amniocentesis	1	3201	Risk Ratio (M-H, Random, 95% CI)	4.95 [0.24, 102.97]

Analysis 3.1. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 1 All known pregnancy loss (including termination of pregnancy).



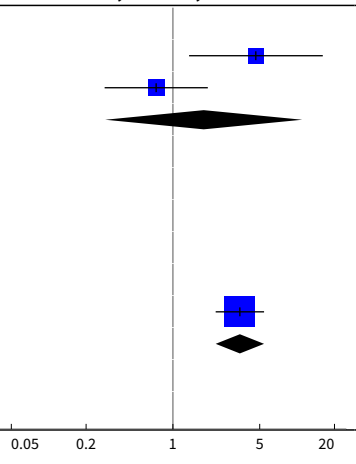
Analysis 3.2. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 2 Spontaneous miscarriage.

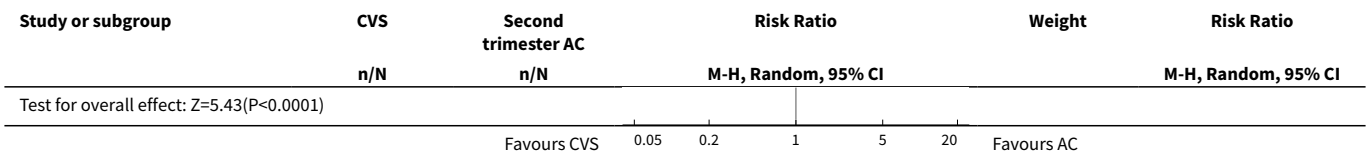
Study or subgroup	CVS	Second trimester AC	Risk Ratio	Weight	Risk Ratio
	n/N	n/N	M-H, Random, 95% CI		M-H, Random, 95% CI
3.2.1 Transcervical CVS versus amniocentesis					
Borrell 1999	75/382	52/400		31.66%	1.51[1.09,2.09]
Canada 1989	196/1348	166/1324		38.84%	1.16[0.96,1.41]
Smidt-Jensen 1993 (Denmark)	83/1010	41/1042		29.5%	2.09[1.45,3.01]
Subtotal (95% CI)	**2740**	**2766**		**100%**	**1.5[1.07,2.11]**
Total events: 354 (CVS), 259 (Second trimester AC)					
Heterogeneity: Tau^2=0.07; Chi^2=8.39, df=2(P=0.02); I^2=76.17%					
Test for overall effect: Z=2.32(P=0.02)					
3.2.2 Transabdominal CVS versus amniocentesis					
Smidt-Jensen 1993 (Denmark)	31/1027	41/1042		100%	0.77[0.49,1.21]
Subtotal (95% CI)	**1027**	**1042**		**100%**	**0.77[0.49,1.21]**
Total events: 31 (CVS), 41 (Second trimester AC)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.13(P=0.26)					
3.2.3 CVS (any route) versus amniocentesis					
MRC 1991	145/1609	92/1592		65.75%	1.56[1.21,2.01]
Smidt-Jensen 1993 (Denmark)	114/2037	41/1042		34.25%	1.42[1,2.02]
Subtotal (95% CI)	**3646**	**2634**		**100%**	**1.51[1.23,1.85]**
Total events: 259 (CVS), 133 (Second trimester AC)					
Heterogeneity: Tau^2=0; Chi^2=0.18, df=1(P=0.67); I^2=0%					
Test for overall effect: Z=3.96(P<0.0001)					



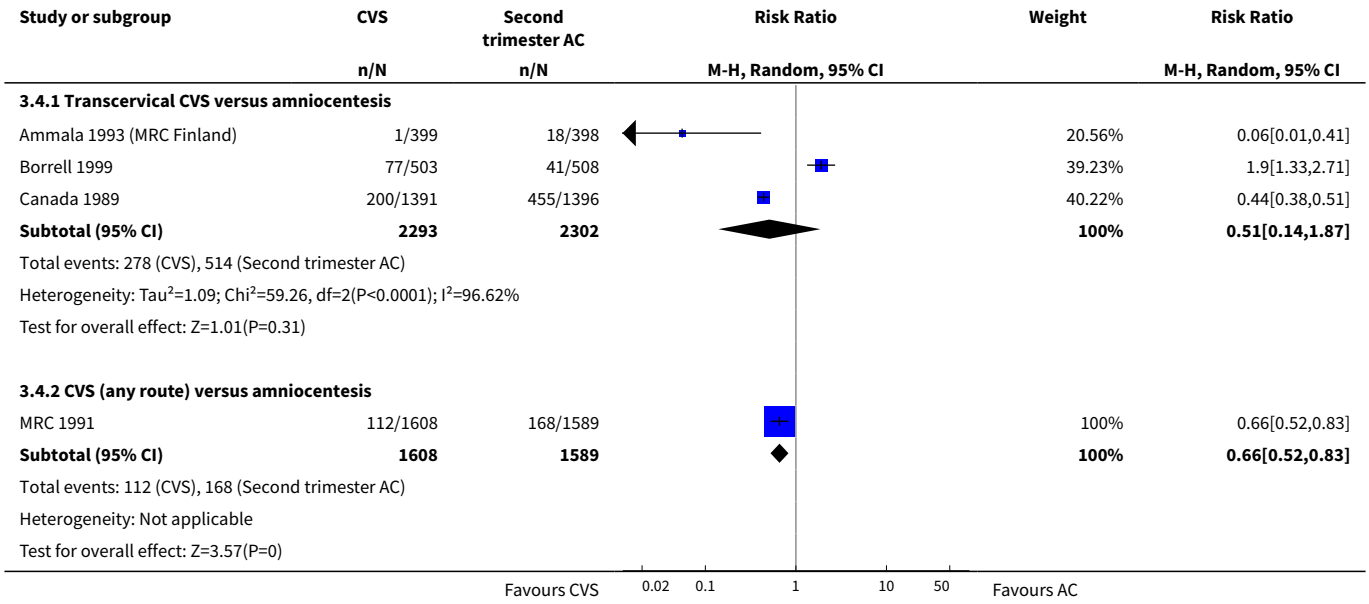
Analysis 3.3. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 3 Spontaneous miscarriage after test.

Study or subgroup	CVS	Second trimester AC	Risk Ratio	Weight	Risk Ratio
	n/N	n/N	M-H, Random, 95% CI		M-H, Random, 95% CI
3.3.1 Transcervical CVS versus amniocentesis					
Ammala 1993 (MRC Finland)	14/399	3/398		47.68%	4.65[1.35,16.07]
Borrell 1999	7/382	10/400		52.32%	0.73[0.28,1.91]
Subtotal (95% CI)	**781**	**798**		**100%**	**1.77[0.28,11]**
Total events: 21 (CVS), 13 (Second trimester AC)					
Heterogeneity: Tau^2=1.42; Chi^2=5.47, df=1(P=0.02); I^2=81.7%					
Test for overall effect: Z=0.61(P=0.54)					
3.3.2 CVS (any route) versus amniocentesis					
MRC 1991	84/1609	24/1592		100%	3.46[2.21,5.42]
Subtotal (95% CI)	**1609**	**1592**		**100%**	**3.46[2.21,5.42]**
Total events: 84 (CVS), 24 (Second trimester AC)					
Heterogeneity: Not applicable					

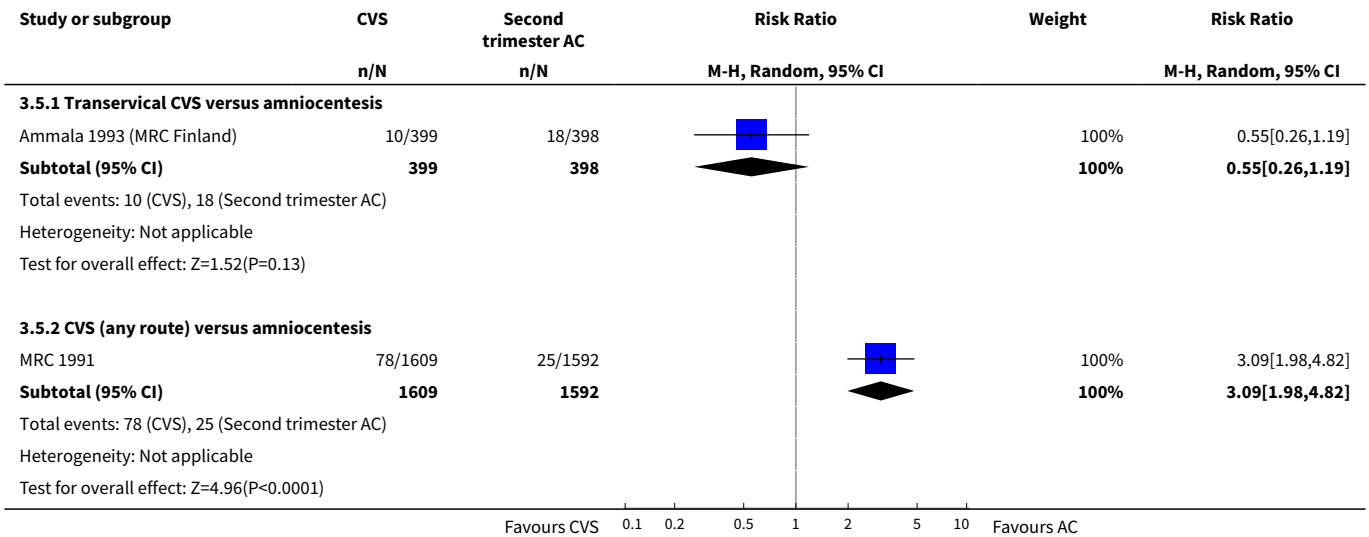




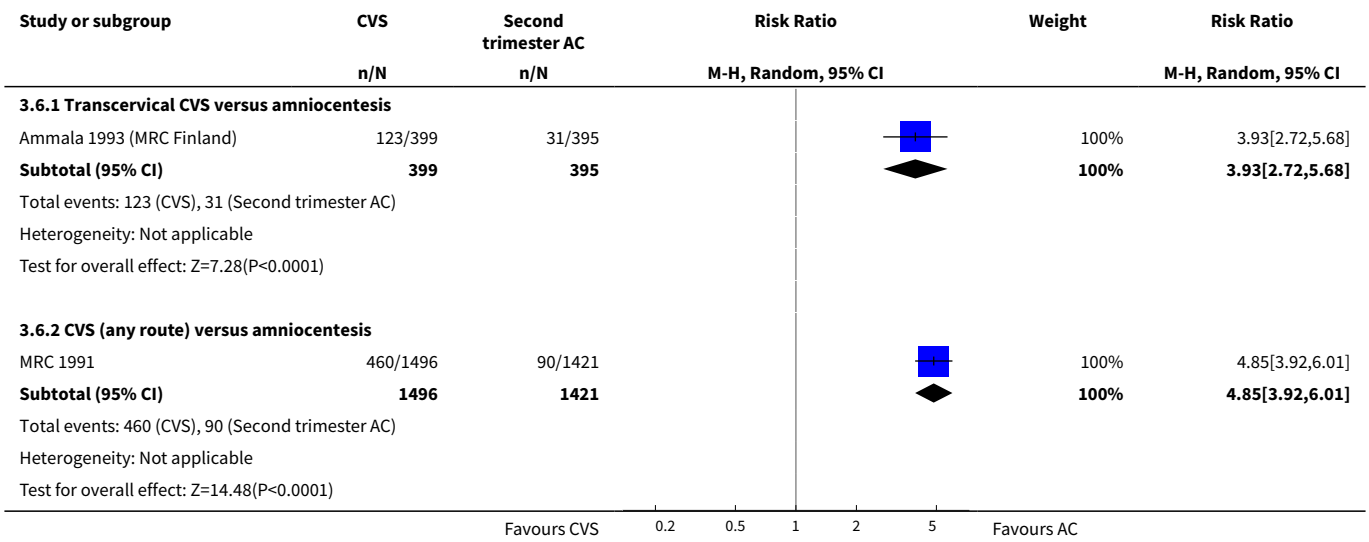
Analysis 3.4. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 4 Non-compliance with allocated procedure.



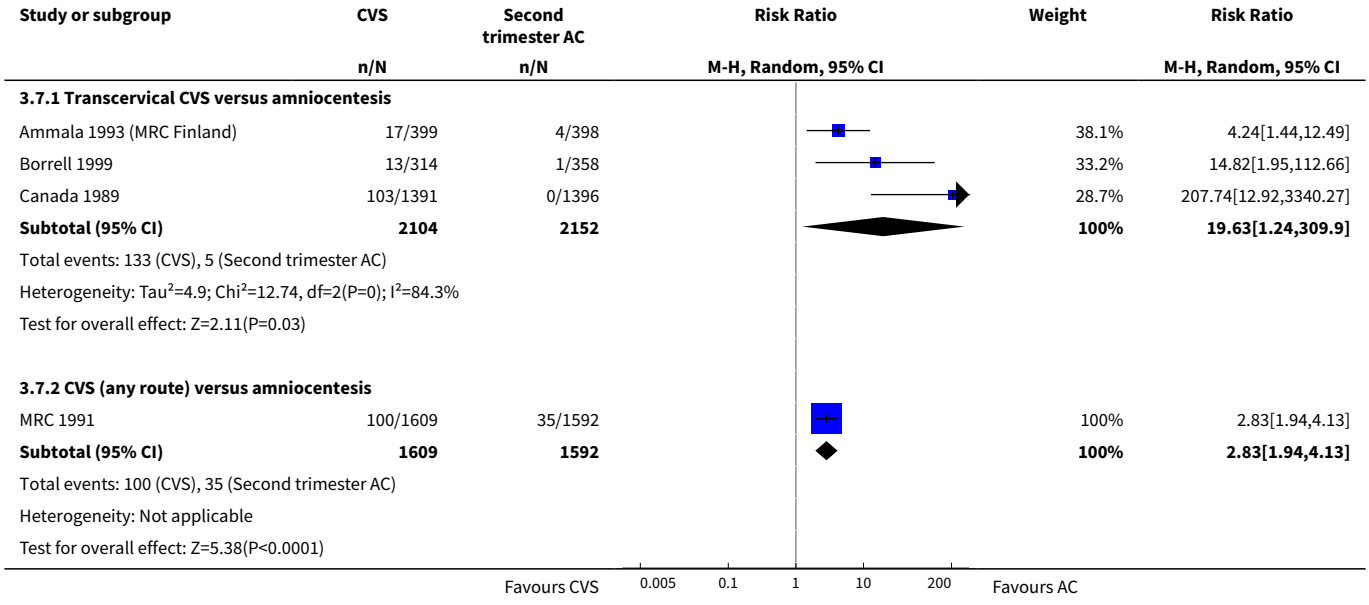
Analysis 3.5. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 5 Sampling failure.



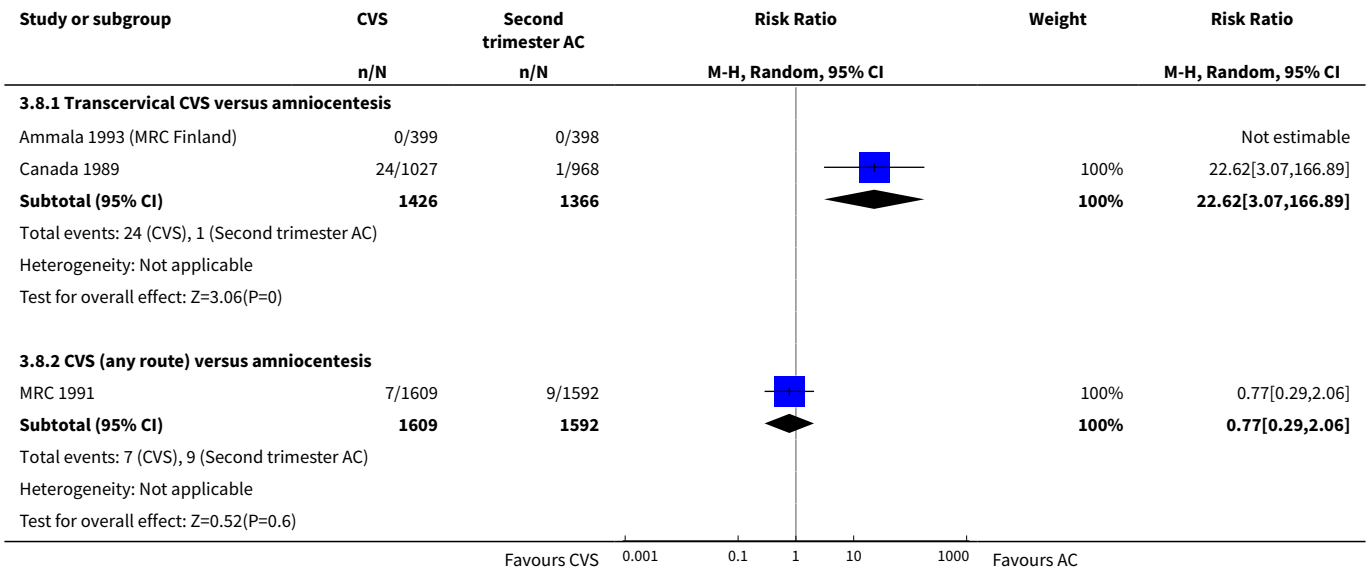
Analysis 3.6. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 6 Multiple insertions.



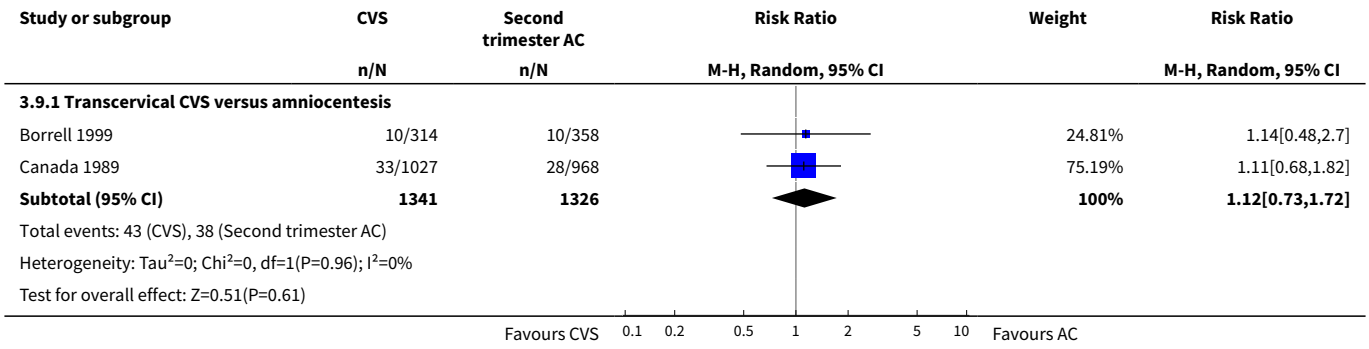
Analysis 3.7. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 7 Second test performed.



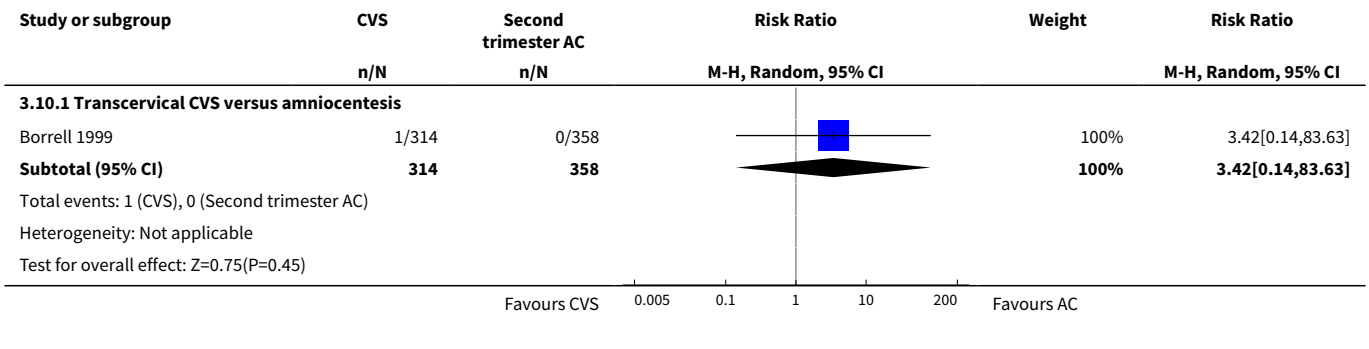
Analysis 3.8. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 8 Laboratory failure.



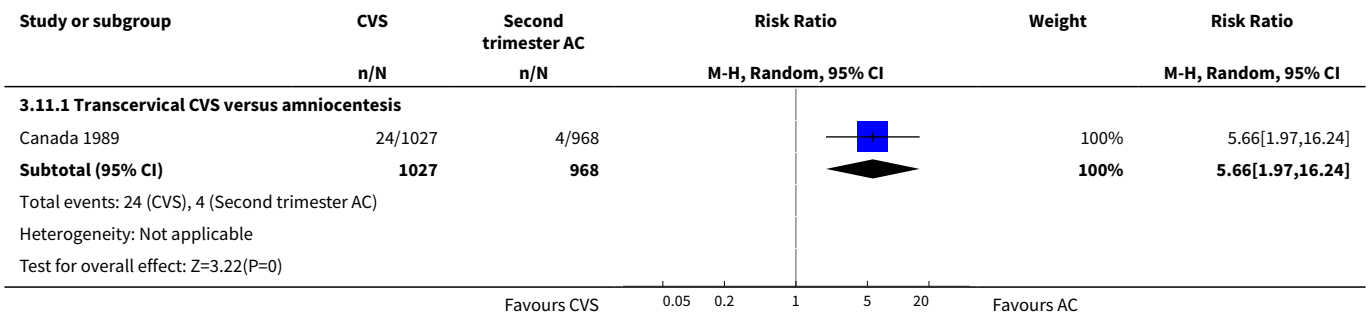
Analysis 3.9. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 9 All non-mosaic abnormalities.



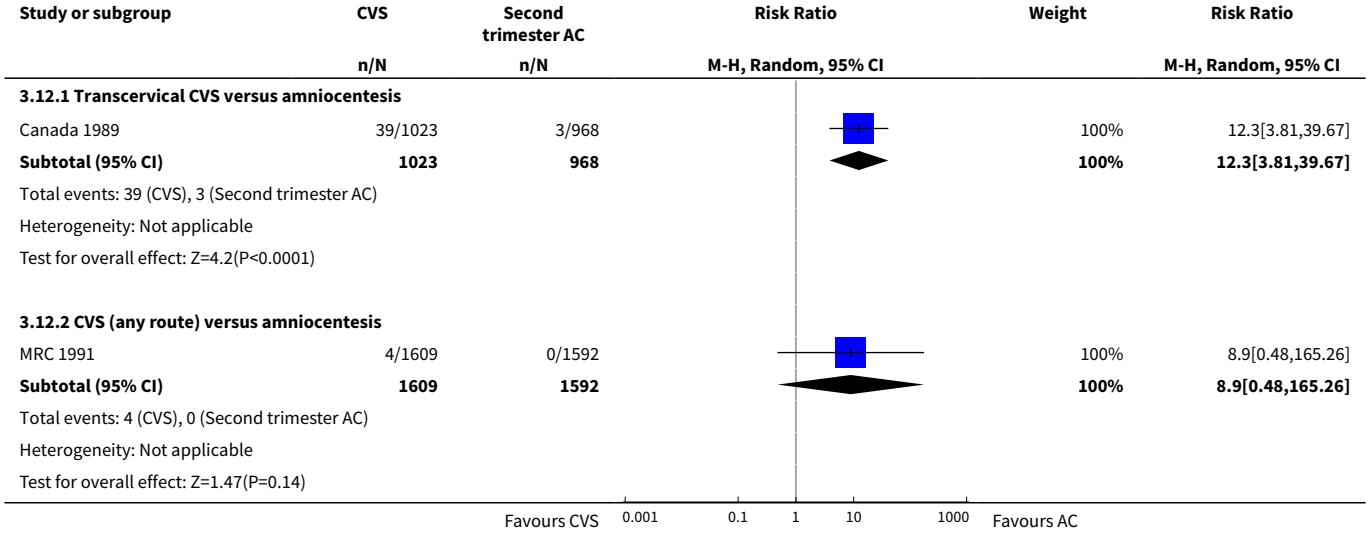
Analysis 3.10. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 10 True mosaics.



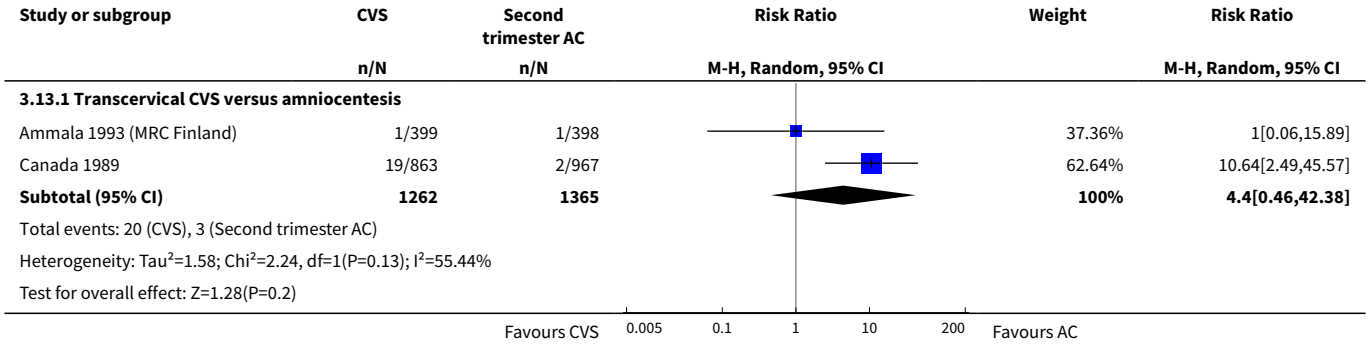
Analysis 3.11. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 11 Confined mosaics.

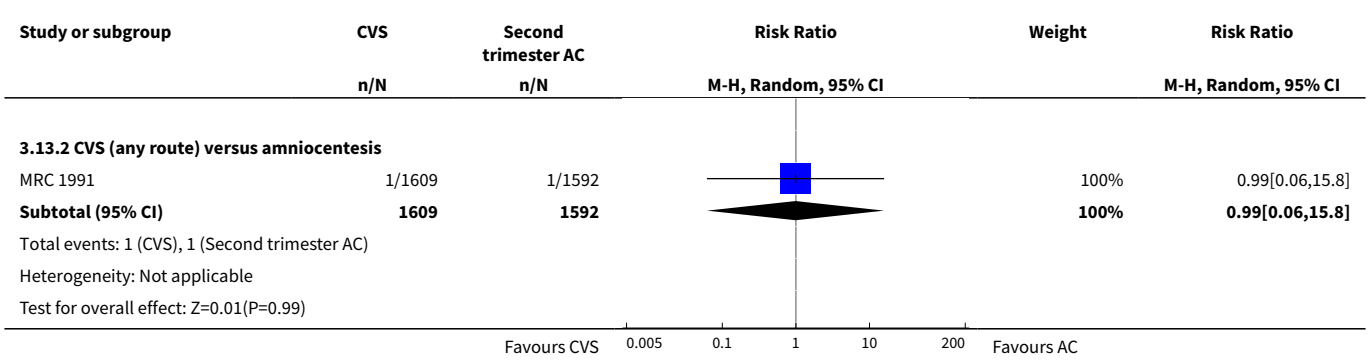


Analysis 3.12. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 12 Maternal contamination.

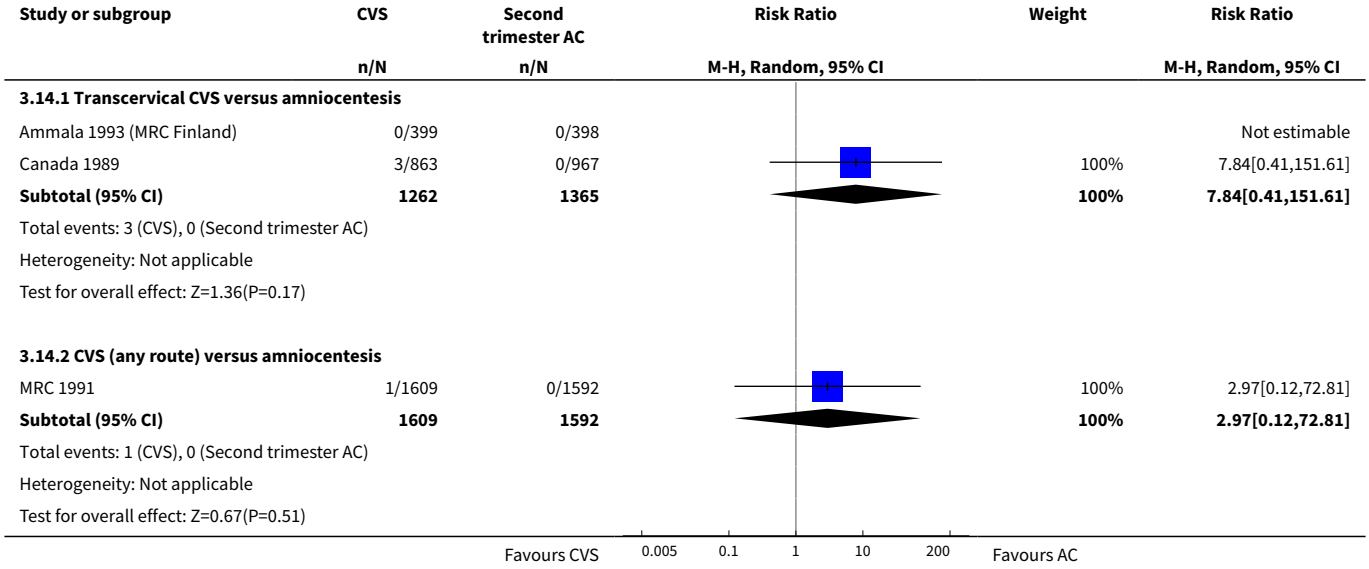


Analysis 3.13. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 13 Known false positive after birth.

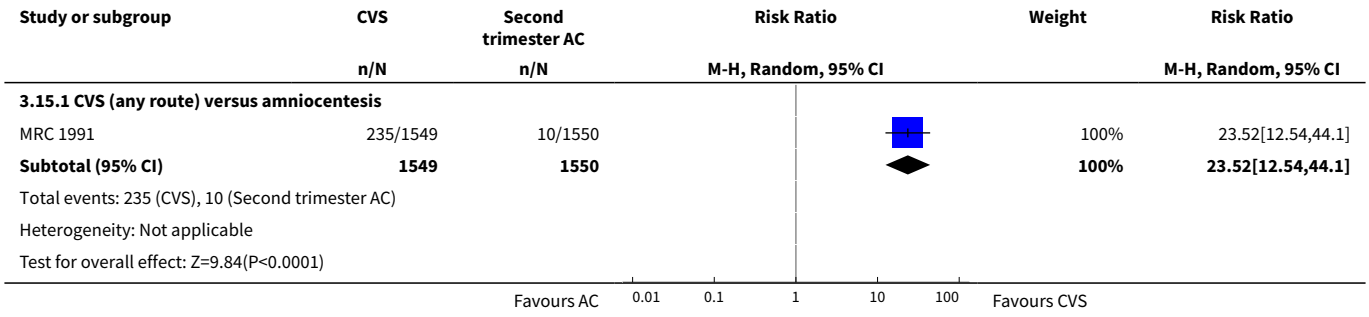




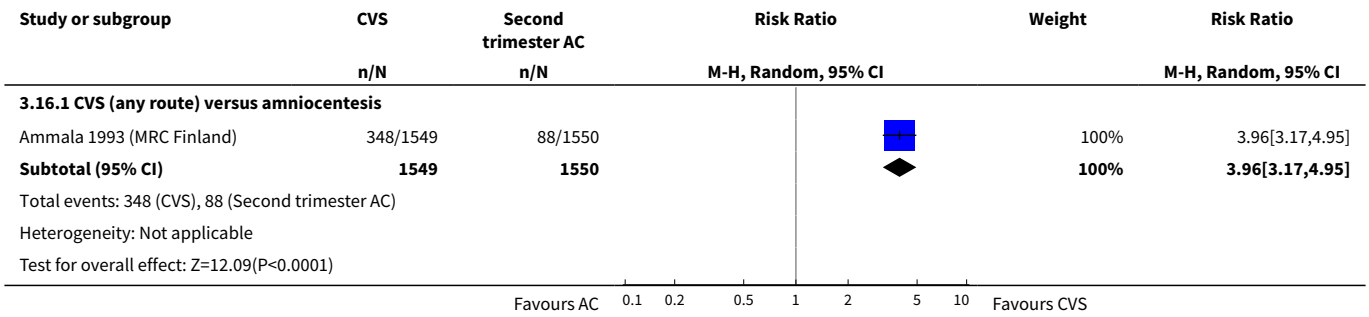
Analysis 3.14. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 14 Known false negative after birth.



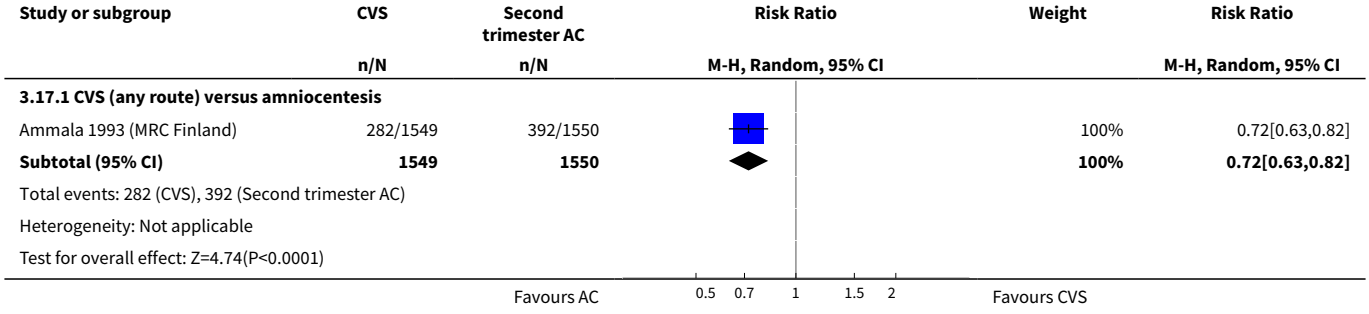
Analysis 3.15. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 15 Results given in less than 7 days (not pre-specified).



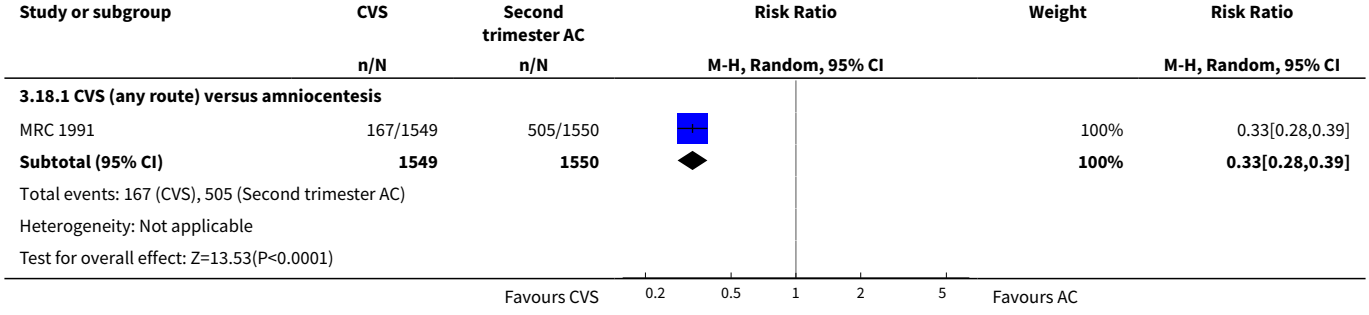
Analysis 3.16. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 16 Results given in less than 14 days (not pre-specified).



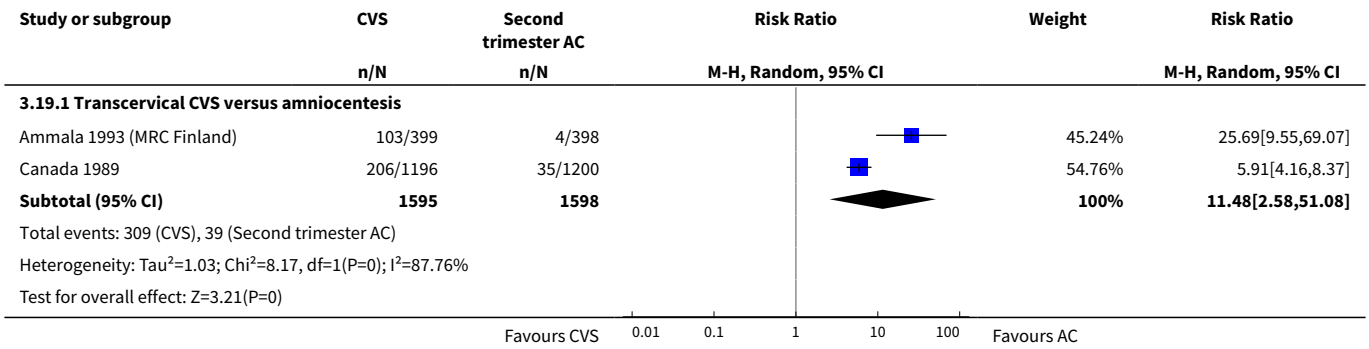
Analysis 3.17. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 17 Results given in less than 21 days (not pre-specified).



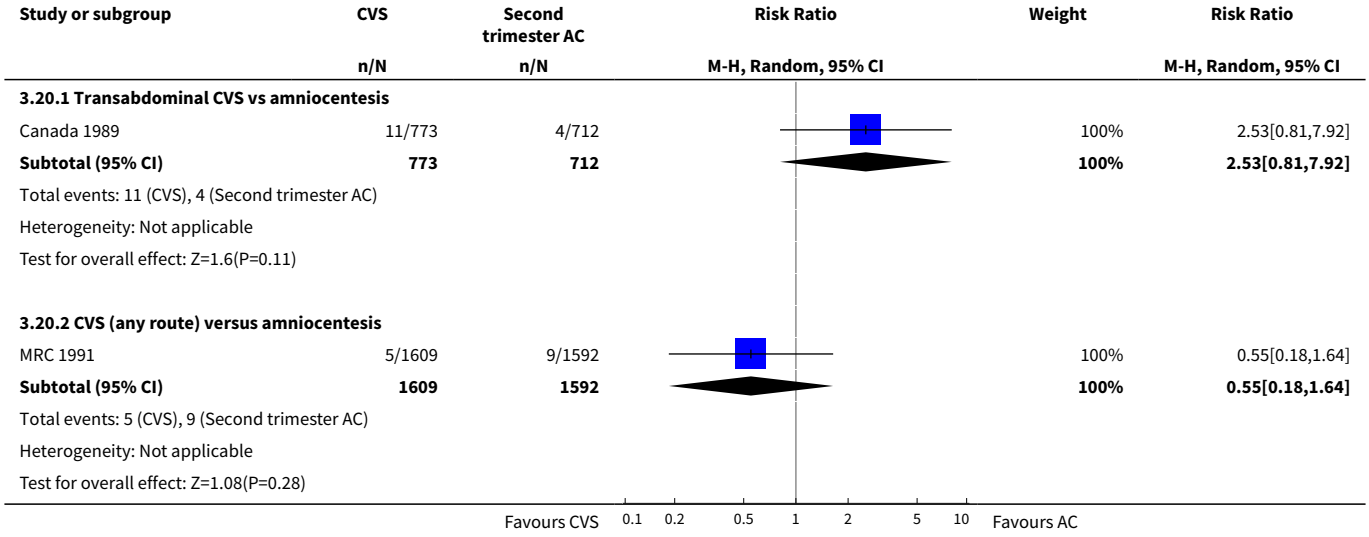
Analysis 3.18. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 18 Result given after 21 days (not pre-specified).



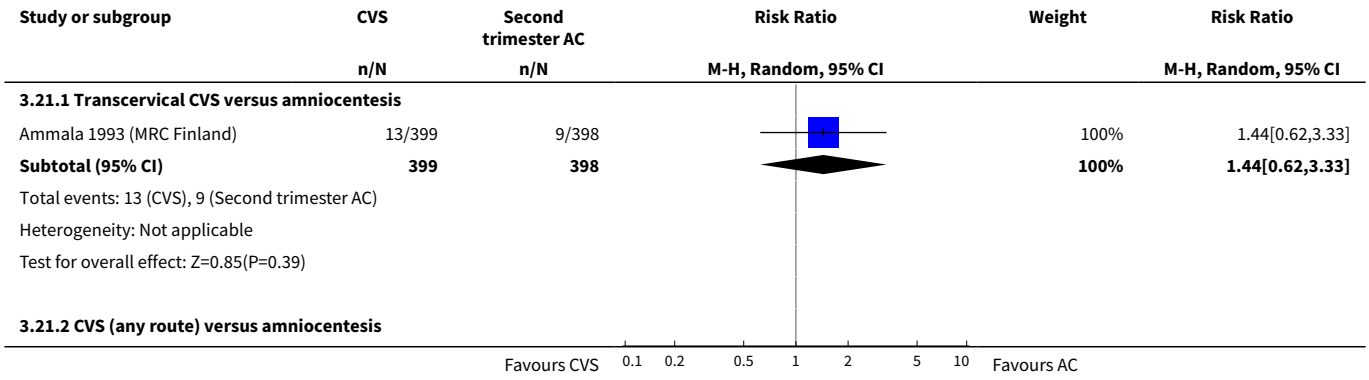
Analysis 3.19. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 19 Vaginal bleeding after test.

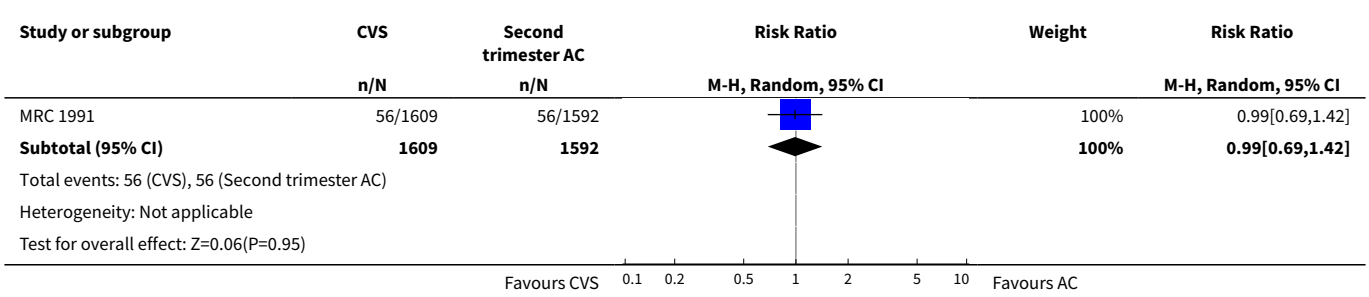


Analysis 3.20. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 20 Amniotic leakage after test.

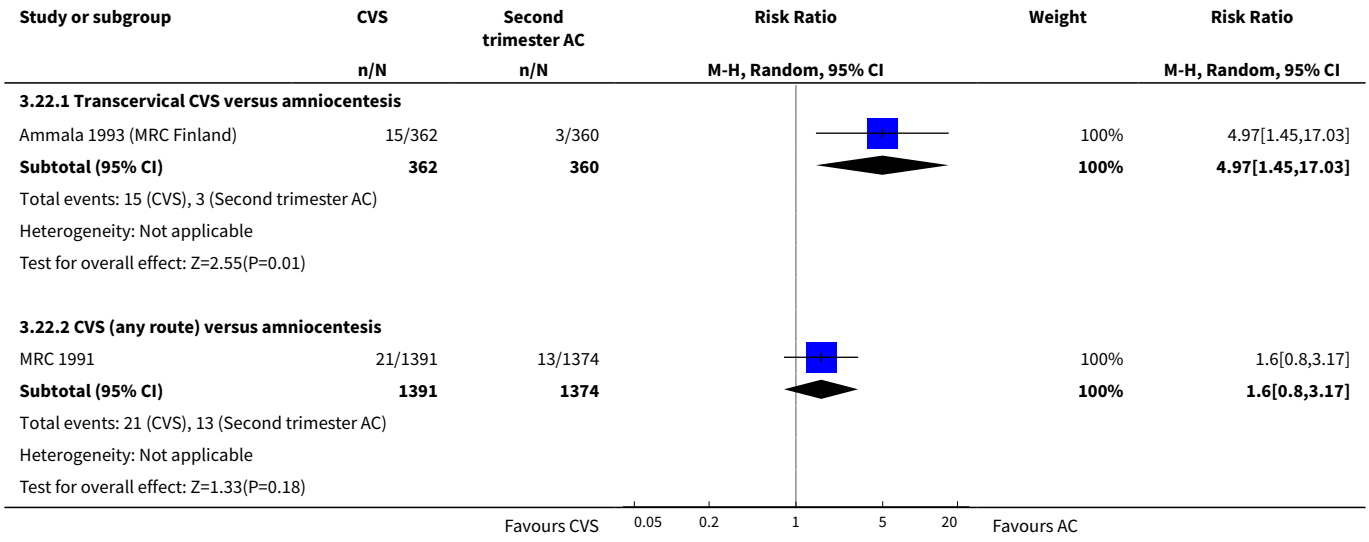


Analysis 3.21. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 21 Vaginal bleeding after 20 weeks.

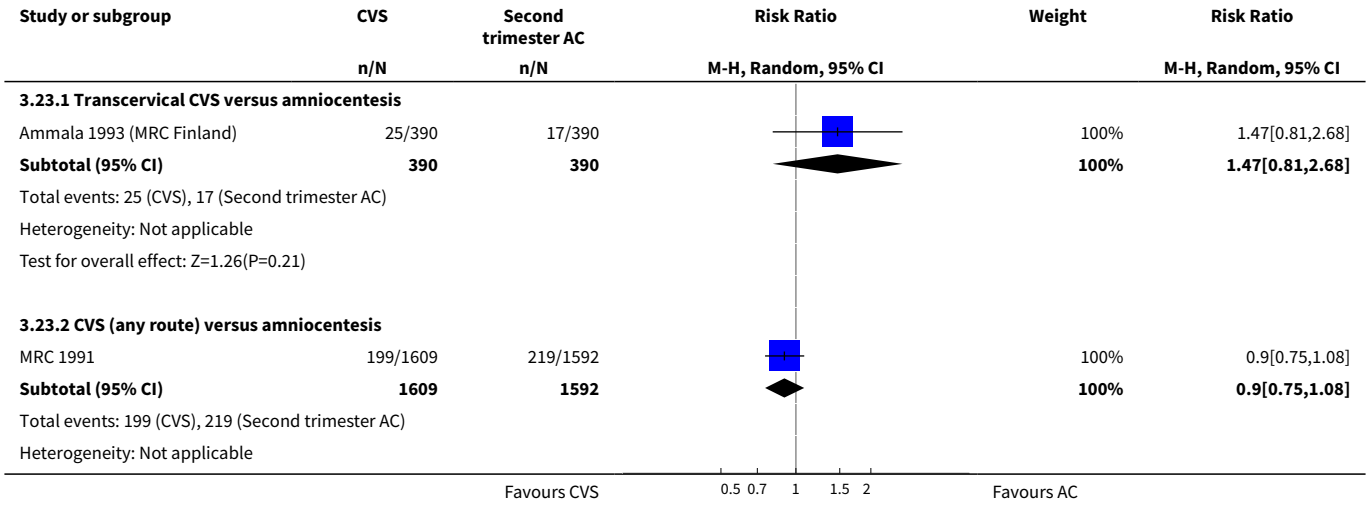


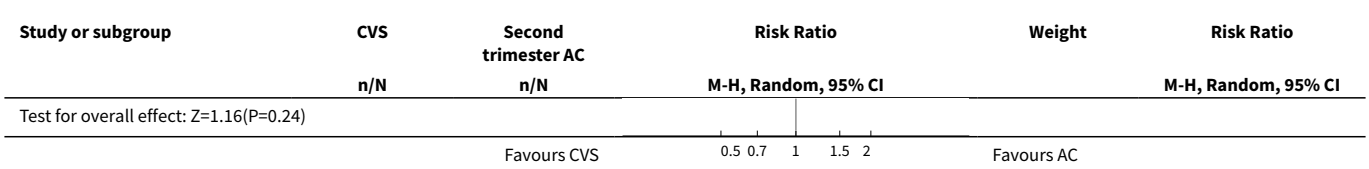


Analysis 3.22. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 22 Pre-labour ruptured membranes before 28 weeks.

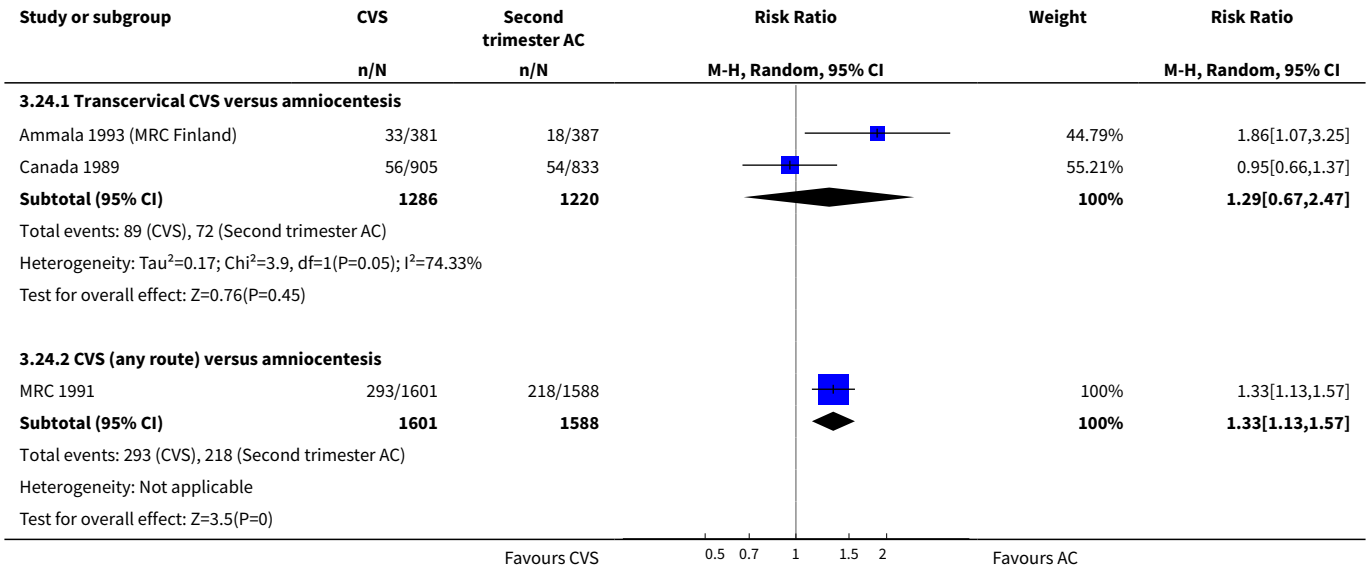


Analysis 3.23. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 23 Antenatal hospital admission.

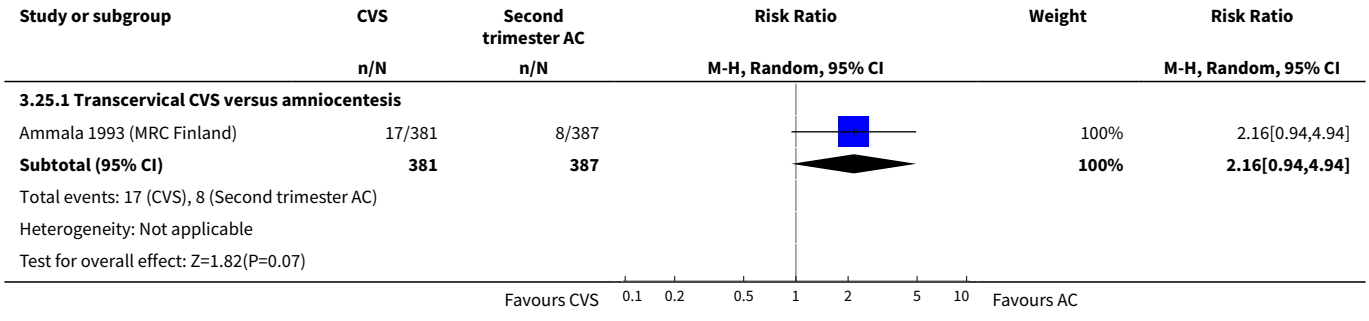




Analysis 3.24. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 24 Delivery before 37 weeks.

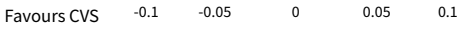


Analysis 3.25. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 25 Delivery before 33 weeks.



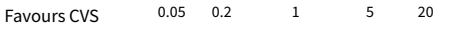
Analysis 3.26. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 26 Termination of pregnancy (all).

Study or subgroup	CVS n/N	Second trimester AC n/N	Risk Difference M-H, Random, 95% CI	Weight	Risk Difference M-H, Random, 95% CI
3.26.1 Transcervical CVS versus amniocentesis					
Borrell 1999	7/382	5/400		36.96%	0.01[-0.01,0.02]
Canada 1989	34/1348	41/1324		63.04%	-0.01[-0.02,0.01]
Subtotal (95% CI)	**1730**	**1724**		**100%**	**-0[-0.01,0.01]**
Total events: 41 (CVS), 46 (Second trimester AC)					
Heterogeneity: Tau^2=0; Chi^2=1.2, df=1(P=0.27); I^2=16.34%					
Test for overall effect: Z=0.26(P=0.8)					
3.26.2 CVS (any route) versus amniocentesis					
MRC 1991	59/1609	41/1592		100%	0.01[-0,0.02]
Subtotal (95% CI)	**1609**	**1592**		**100%**	**0.01[-0,0.02]**
Total events: 59 (CVS), 41 (Second trimester AC)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.78(P=0.08)					

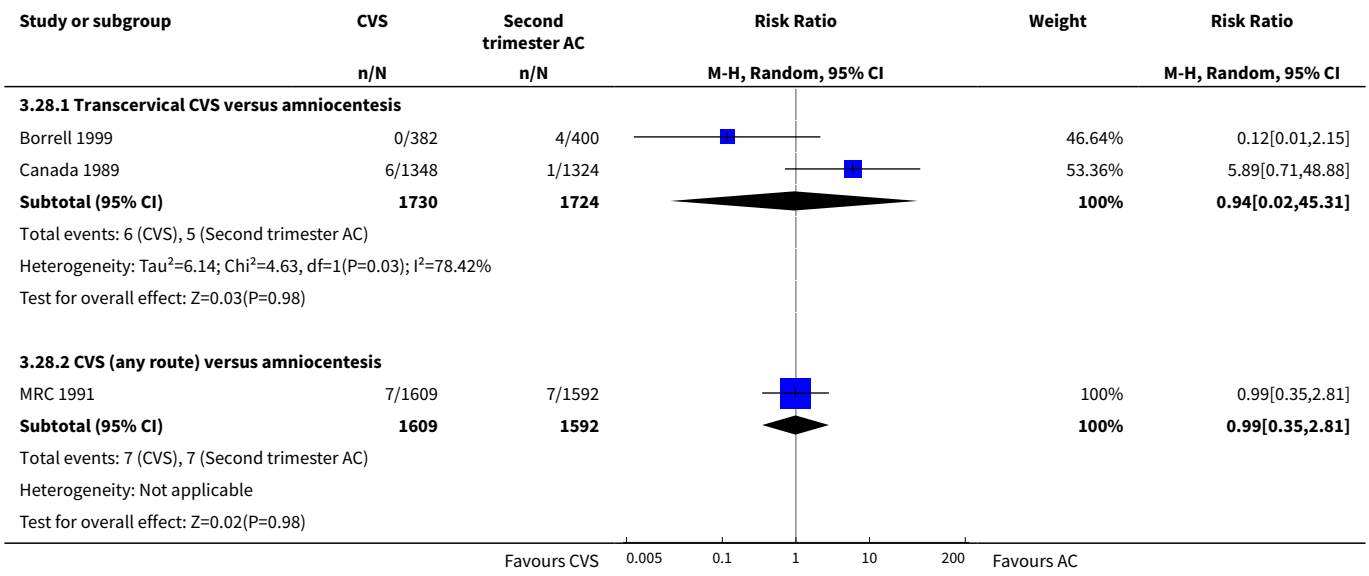


Analysis 3.27. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 27 Perinatal deaths.

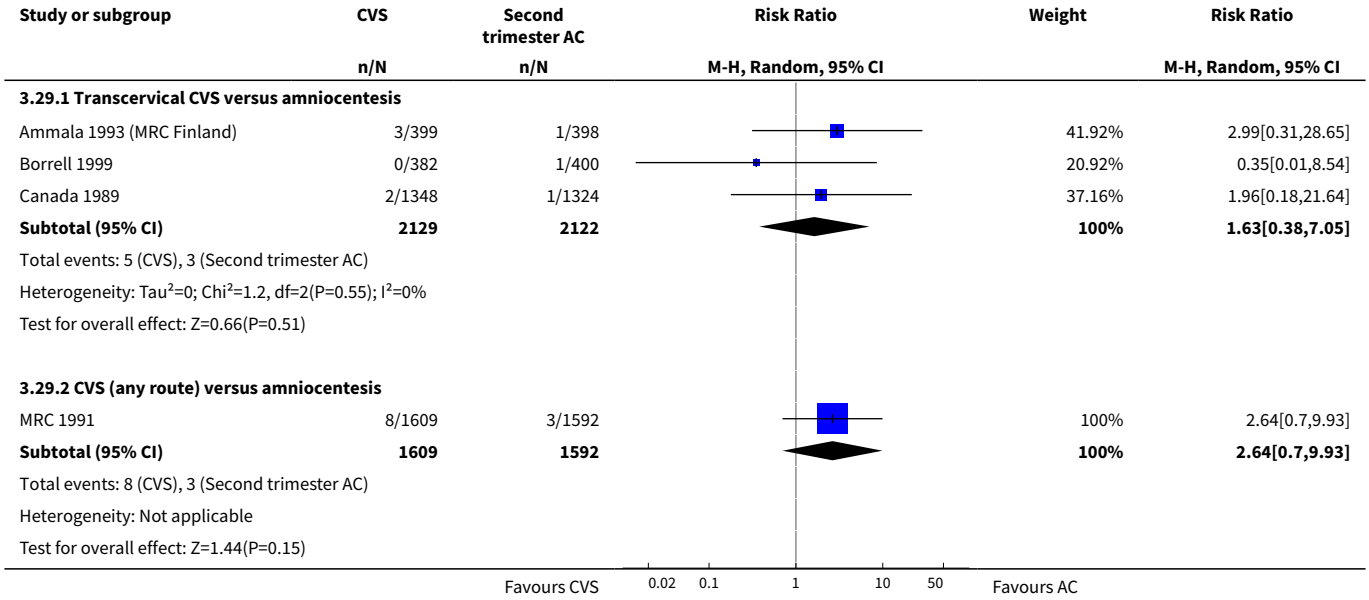
Study or subgroup	CVS n/N	Second trimester AC n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
3.27.1 Transcervical CVS versus amniocentesis					
Ammala 1993 (MRC Finland)	4/399	1/398		25.46%	3.99[0.45,35.54]
Canada 1989	8/1348	2/1324		35.69%	3.93[0.84,18.47]
Smidt-Jensen 1993 (Denmark)	3/1010	6/1042		38.85%	0.52[0.13,2.06]
Subtotal (95% CI)	**2757**	**2764**		**100%**	**1.79[0.42,7.69]**
Total events: 15 (CVS), 9 (Second trimester AC)					
Heterogeneity: Tau^2=0.92; Chi^2=4.56, df=2(P=0.1); I^2=56.11%					
Test for overall effect: Z=0.79(P=0.43)					
3.27.2 Transabdominal CVS versus amniocentesis					
Smidt-Jensen 1993 (Denmark)	7/1027	6/1042		100%	1.18[0.4,3.51]
Subtotal (95% CI)	**1027**	**1042**		**100%**	**1.18[0.4,3.51]**
Total events: 7 (CVS), 6 (Second trimester AC)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.3(P=0.76)					
3.27.3 CVS (any route) versus amniocentesis					
MRC 1991	15/1609	10/1592		61.59%	1.48[0.67,3.29]
Smidt-Jensen 1993 (Denmark)	10/2037	6/1042		38.41%	0.85[0.31,2.34]
Subtotal (95% CI)	**3646**	**2634**		**100%**	**1.2[0.64,2.24]**
Total events: 25 (CVS), 16 (Second trimester AC)					
Heterogeneity: Tau^2=0; Chi^2=0.71, df=1(P=0.4); I^2=0%					
Test for overall effect: Z=0.57(P=0.57)					



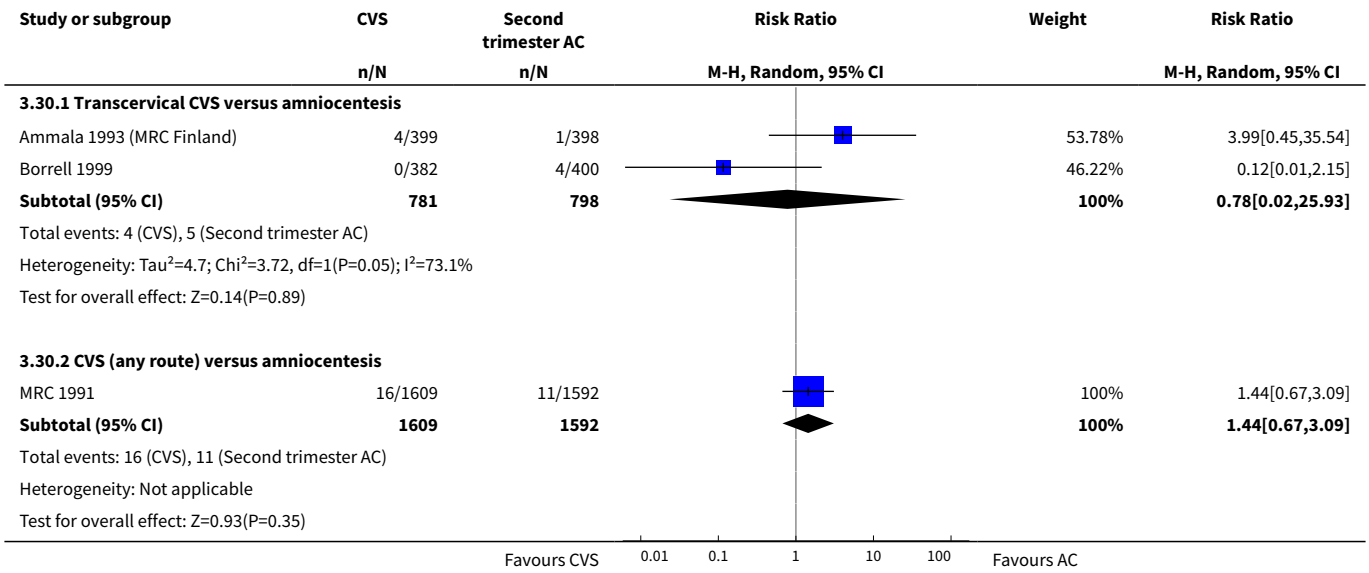
Analysis 3.28. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 28 Stillbirths.



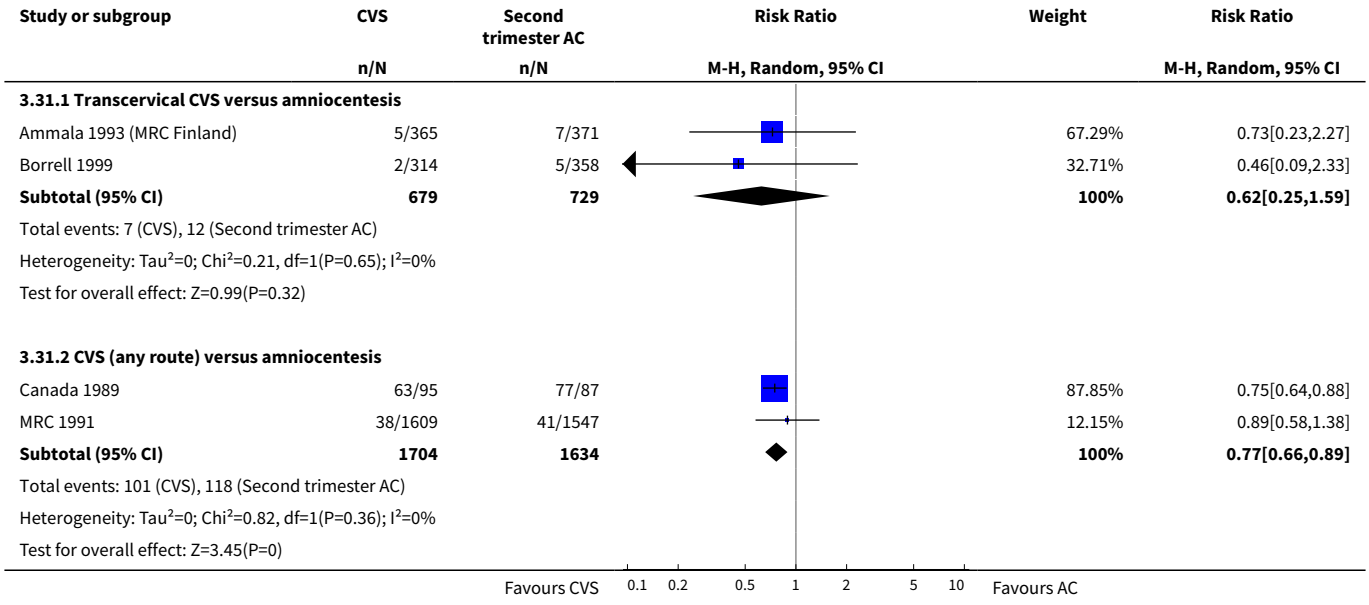
Analysis 3.29. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 29 Neonatal deaths.



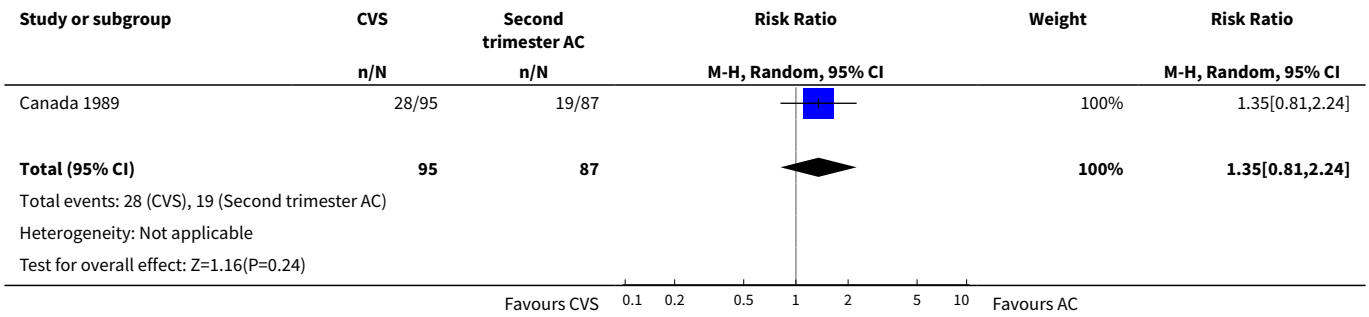
Analysis 3.30. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 30 All recorded deaths after viability.



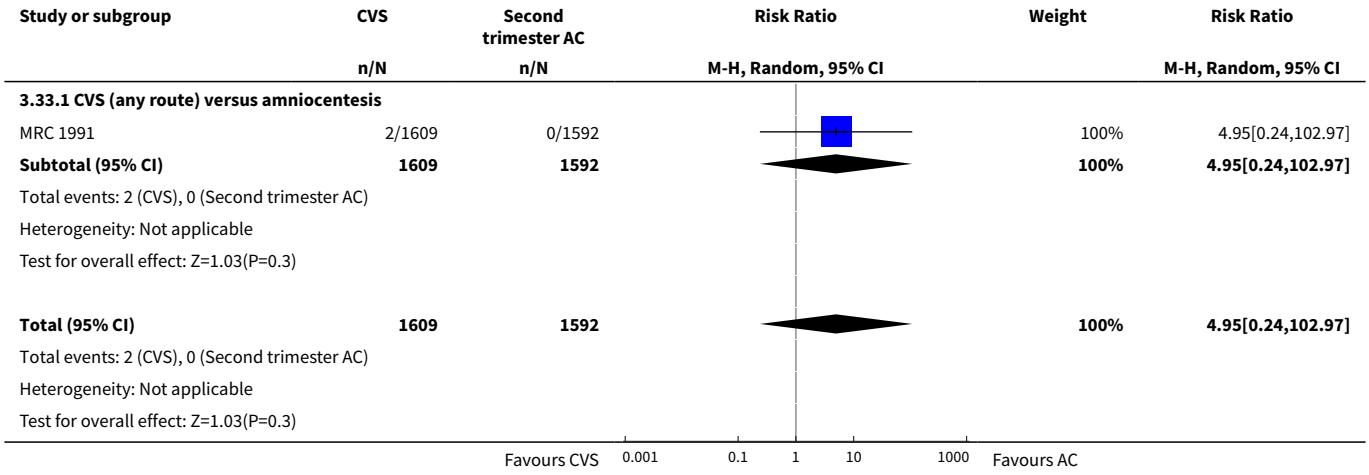
Analysis 3.31. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 31 Congenital anomalies (all recorded).



Analysis 3.32. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 32 Haemangioma.



Analysis 3.33. Comparison 3 Chorionic villus sampling (CVS) versus second trimester amniocentesis (AC), Outcome 33 Limb reduction defects.

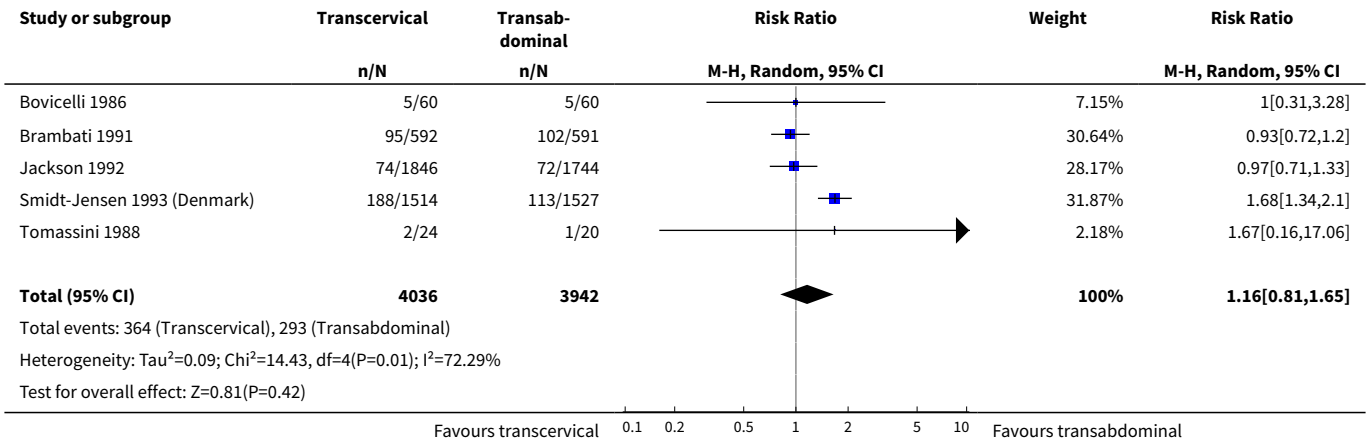


Comparison 4. Transcervical versus transabdominal chorionic villus sampling (CVS)

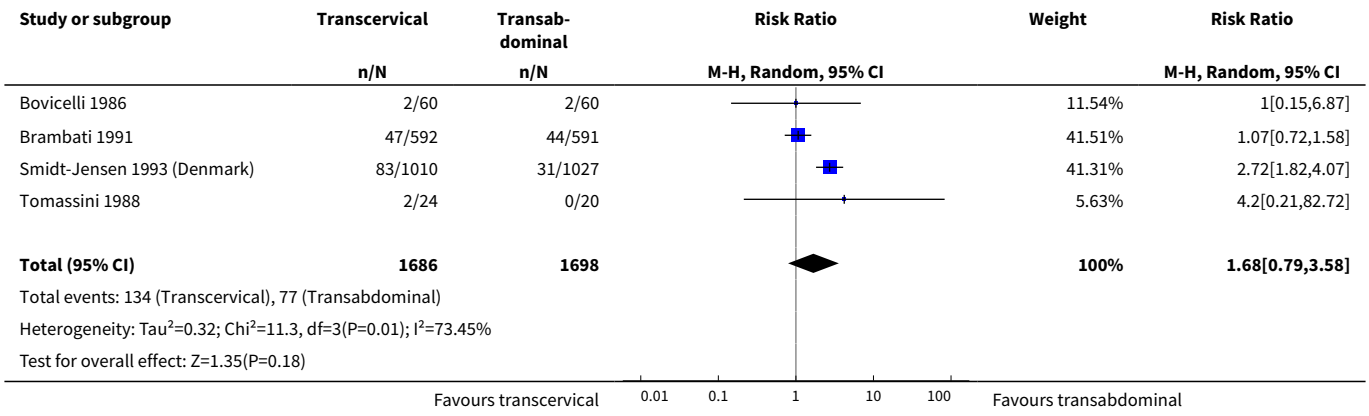
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All known pregnancy loss (including termination of pregnancy)	5	7978	Risk Ratio (M-H, Random, 95% CI)	1.16 [0.81, 1.65]
2 Spontaneous miscarriage	4	3384	Risk Ratio (M-H, Random, 95% CI)	1.68 [0.79, 3.58]
3 Spontaneous miscarriage after test	3	1347	Risk Ratio (M-H, Random, 95% CI)	1.23 [0.75, 2.04]
4 Non-compliance with allocated procedure	3	5187	Risk Ratio (M-H, Random, 95% CI)	1.68 [0.59, 4.76]
5 Sampling failure	4	5231	Risk Ratio (M-H, Random, 95% CI)	1.79 [1.13, 2.82]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6 Multiple insertions	2	1314	Risk Ratio (M-H, Random, 95% CI)	2.54 [1.47, 4.42]
7 Second test performed	1	1194	Risk Ratio (M-H, Random, 95% CI)	1.24 [0.65, 2.37]
8 Laboratory failure	1	1194	Risk Ratio (M-H, Random, 95% CI)	2.23 [0.69, 7.22]
9 All non-mosaic abnormalities	1	2862	Risk Ratio (M-H, Random, 95% CI)	1.23 [0.87, 1.75]
10 True mosaics	1	2862	Risk Ratio (M-H, Random, 95% CI)	0.92 [0.39, 2.17]
11 Confined mosaics	1	2862	Risk Ratio (M-H, Random, 95% CI)	0.85 [0.26, 2.77]
12 Amniotic leakage after test	1	44	Risk Ratio (M-H, Random, 95% CI)	0.28 [0.01, 6.52]
13 Vaginal bleeding after test	3	1358	Risk Ratio (M-H, Random, 95% CI)	6.93 [0.77, 62.83]
14 Termination of pregnancy (all)	2	1303	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.56, 1.22]
15 Perinatal deaths	1	2037	Risk Ratio (M-H, Random, 95% CI)	0.44 [0.11, 1.68]
16 Stillbirths	2	1227	Risk Ratio (M-H, Random, 95% CI)	1.36 [0.11, 17.53]
17 Neonatal deaths	2	4845	Risk Ratio (M-H, Random, 95% CI)	0.60 [0.14, 2.55]
18 Anomalies (all recorded)	2	3622	Risk Ratio (M-H, Random, 95% CI)	0.68 [0.41, 1.12]
19 Talipes	1	2624	Risk Ratio (M-H, Random, 95% CI)	3.21 [0.33, 30.80]

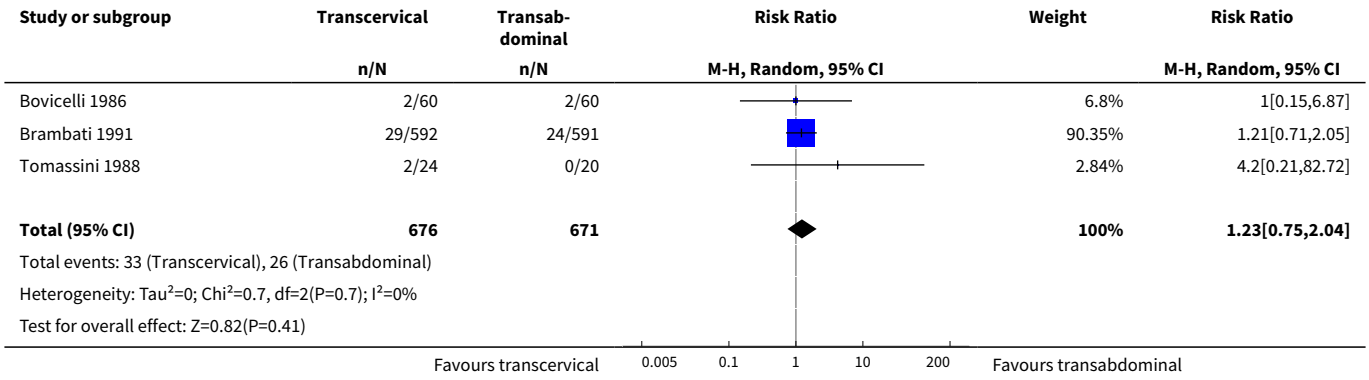
Analysis 4.1. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 1 All known pregnancy loss (including termination of pregnancy).



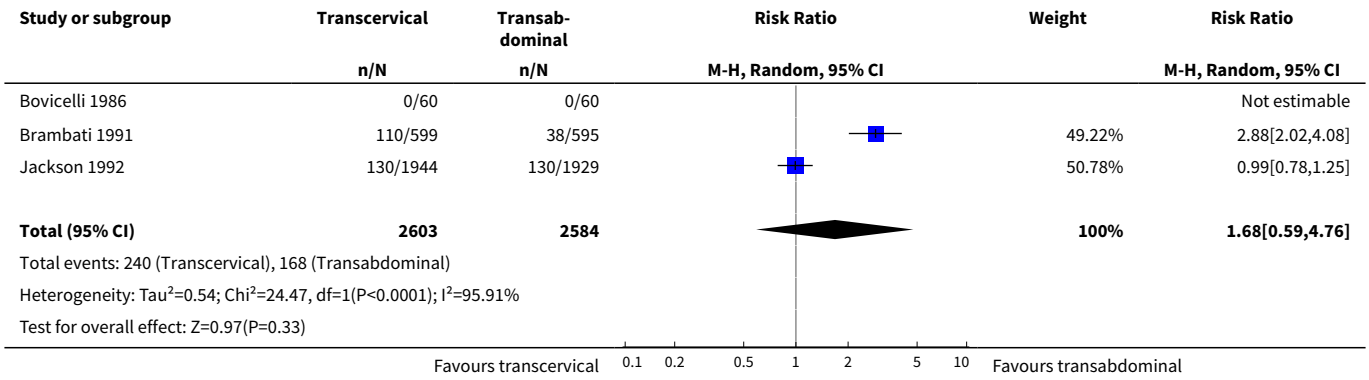
Analysis 4.2. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 2 Spontaneous miscarriage.



Analysis 4.3. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 3 Spontaneous miscarriage after test.



Analysis 4.4. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 4 Non-compliance with allocated procedure.



Analysis 4.5. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 5 Sampling failure.

Study or subgroup	Transcervical n/N	Transabdominal n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Bovicelli 1986	1/60	1/60		2.75%	1[0.06,15.62]
Brambati 1991	1/599	1/595		2.71%	0.99[0.06,15.84]
Jackson 1992	47/1944	26/1929		92.08%	1.79[1.12,2.88]
Tomassini 1988	3/24	0/20		2.46%	5.88[0.32,107.49]
Total (95% CI)	**2627**	**2604**		**100%**	**1.79[1.13,2.82]**
Total events: 52 (Transcervical), 28 (Transabdominal)					
Heterogeneity: Tau^2=0; Chi^2=0.99, df=3(P=0.8); I^2=0%					
Test for overall effect: Z=2.5(P=0.01)					

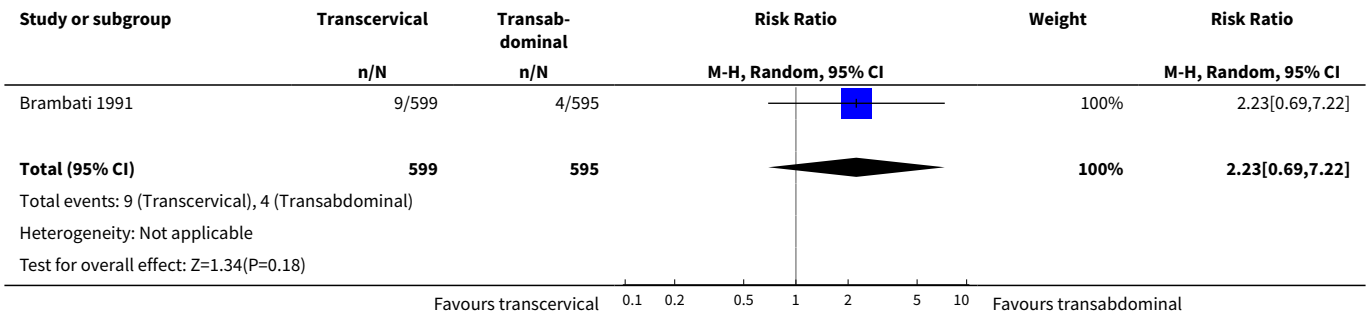
Analysis 4.6. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 6 Multiple insertions.

Study or subgroup	Transcervical n/N	Transabdominal n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Bovicelli 1986	14/60	8/60		35.9%	1.75[0.79,3.86]
Brambati 1991	60/599	19/595		64.1%	3.14[1.9,5.19]
Total (95% CI)	**659**	**655**		**100%**	**2.54[1.47,4.42]**
Total events: 74 (Transcervical), 27 (Transabdominal)					
Heterogeneity: Tau^2=0.06; Chi^2=1.5, df=1(P=0.22); I^2=33.49%					
Test for overall effect: Z=3.32(P=0)					

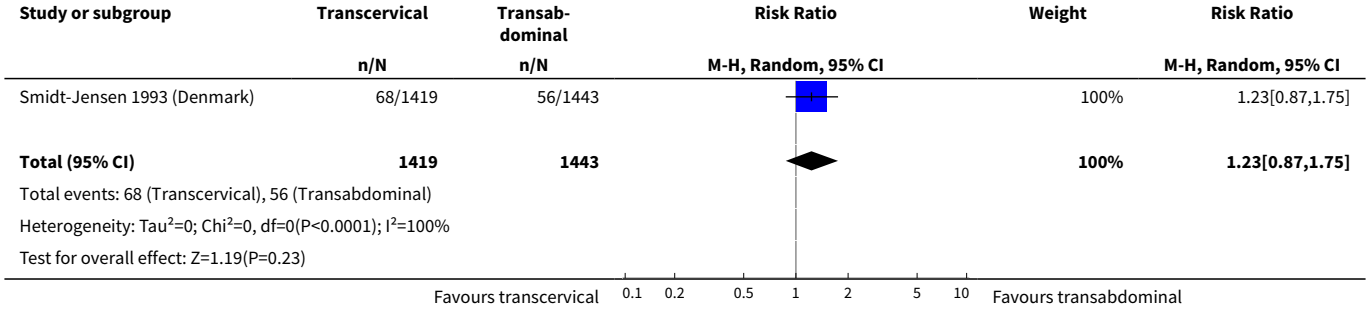
Analysis 4.7. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 7 Second test performed.

Study or subgroup	Transcervical n/N	Transabdominal n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Brambati 1991	20/599	16/595		100%	1.24[0.65,2.37]
Total (95% CI)	**599**	**595**		**100%**	**1.24[0.65,2.37]**
Total events: 20 (Transcervical), 16 (Transabdominal)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.66(P=0.51)					

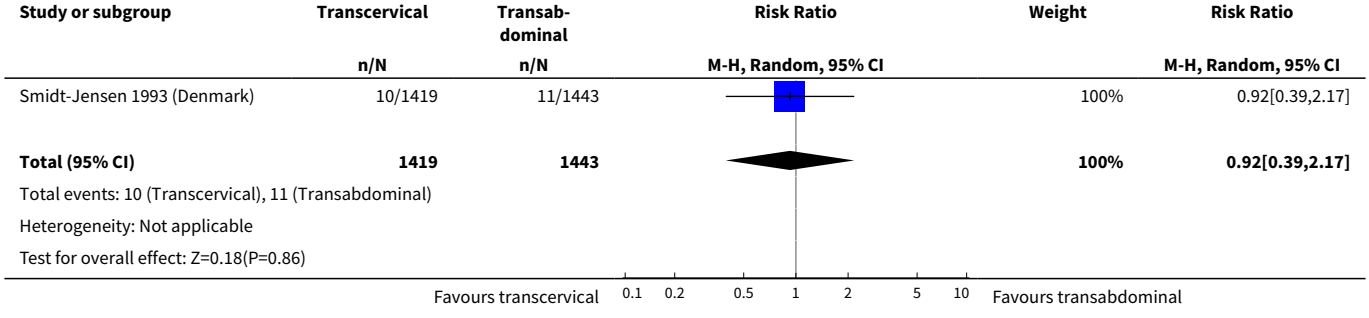
Analysis 4.8. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 8 Laboratory failure.



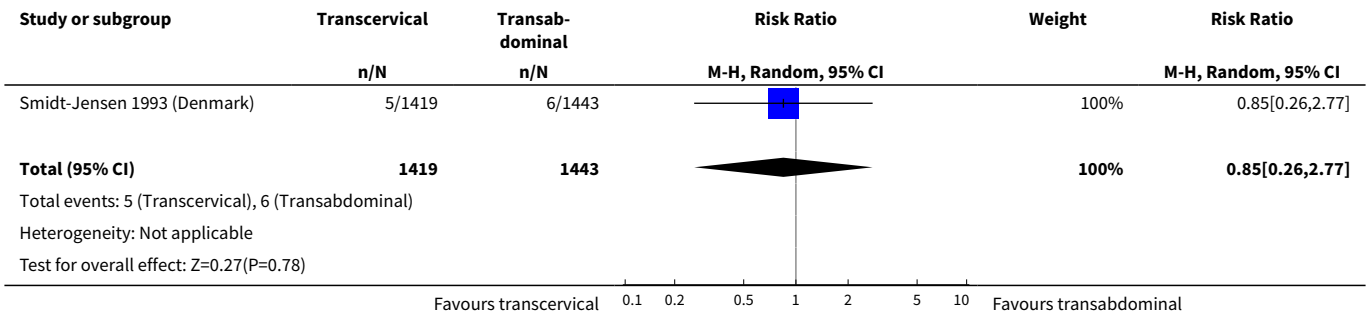
Analysis 4.9. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 9 All non-mosaic abnormalities.



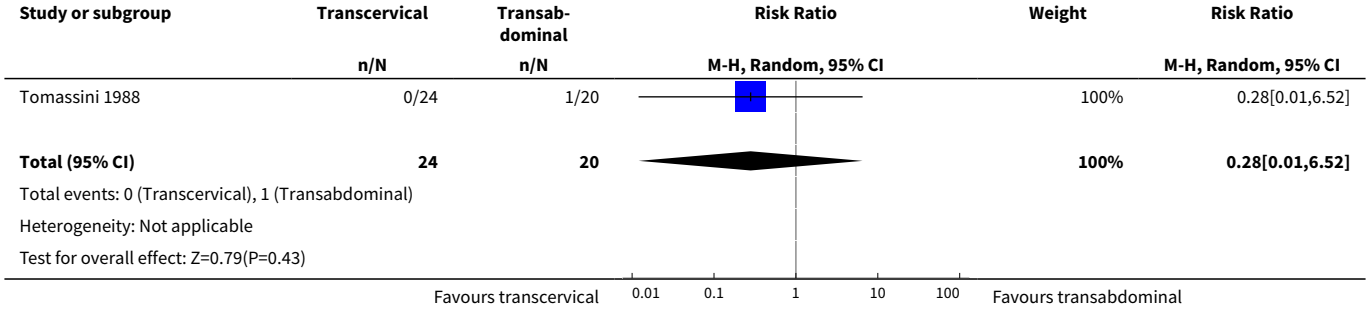
Analysis 4.10. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 10 True mosaics.



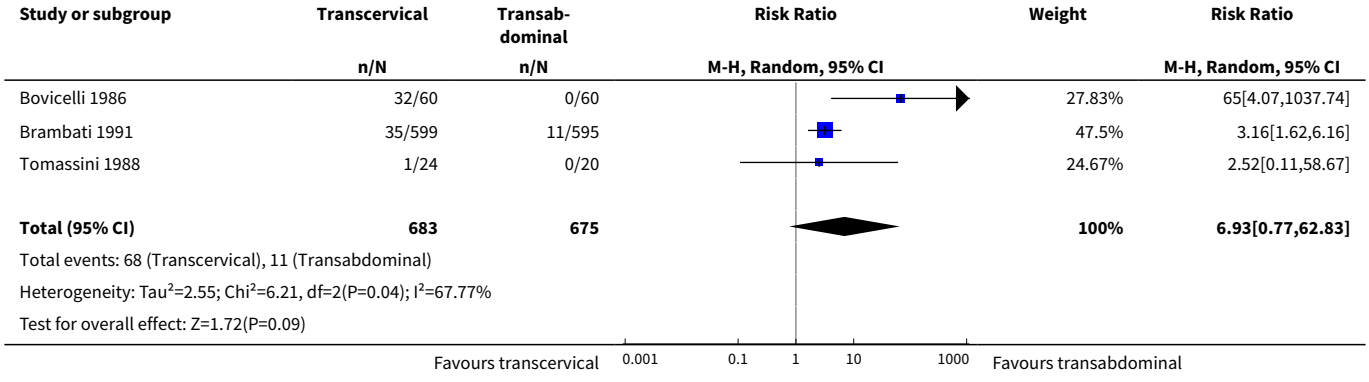
Analysis 4.11. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 11 Confined mosaics.



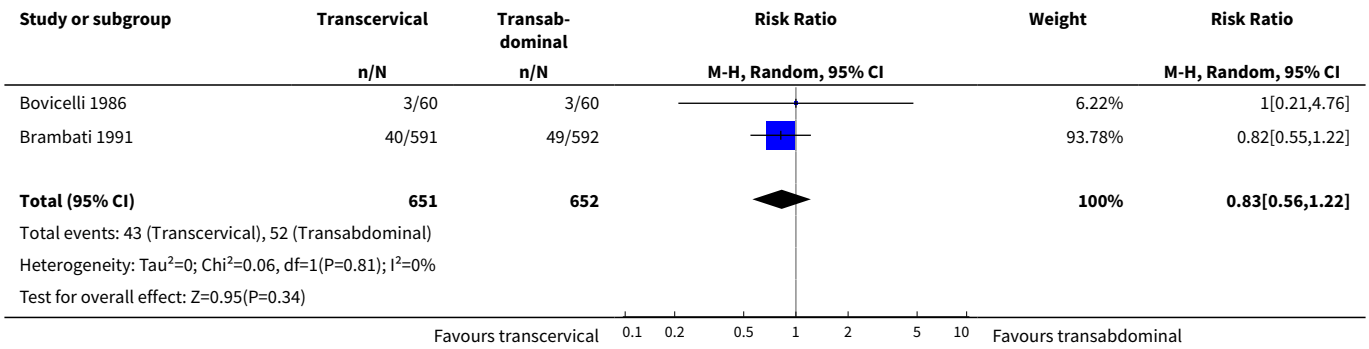
Analysis 4.12. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 12 Amniotic leakage after test.



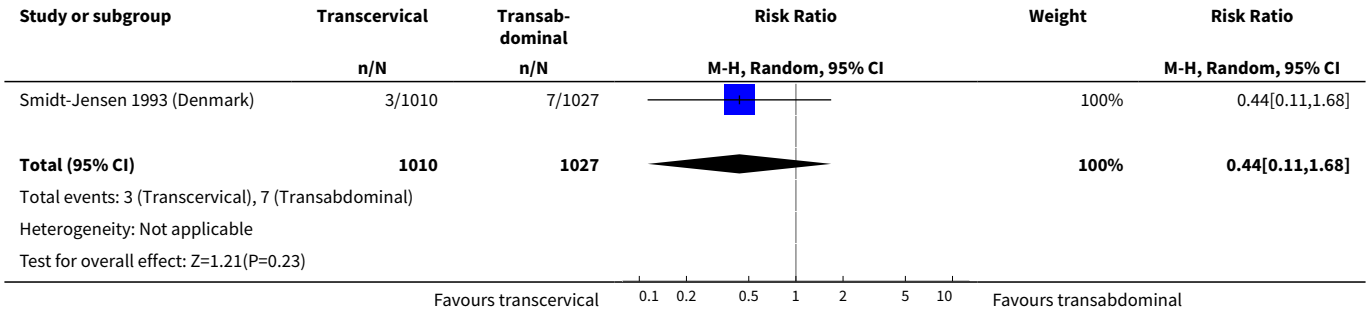
Analysis 4.13. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 13 Vaginal bleeding after test.



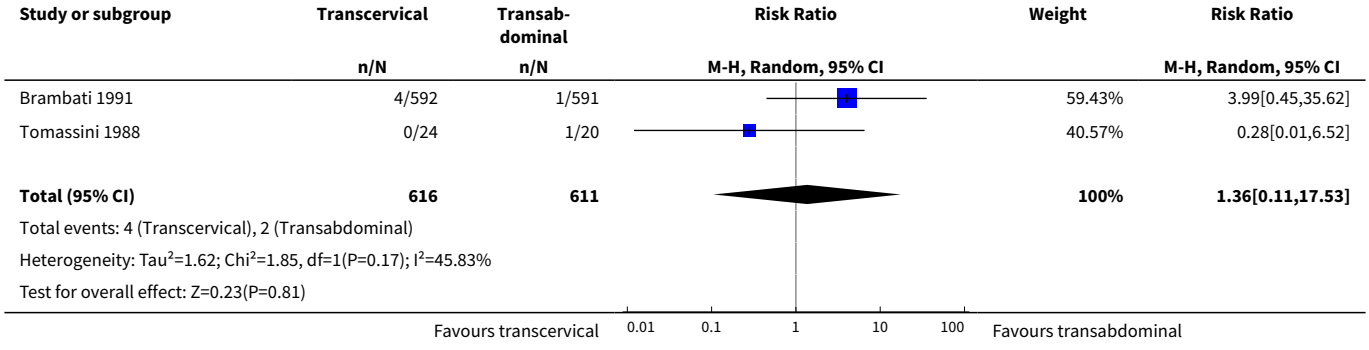
Analysis 4.14. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 14 Termination of pregnancy (all).



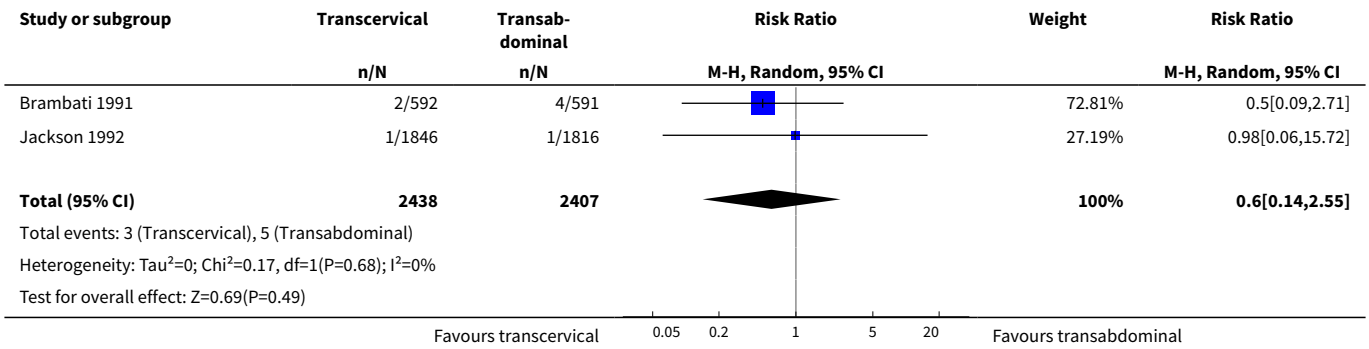
Analysis 4.15. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 15 Perinatal deaths.



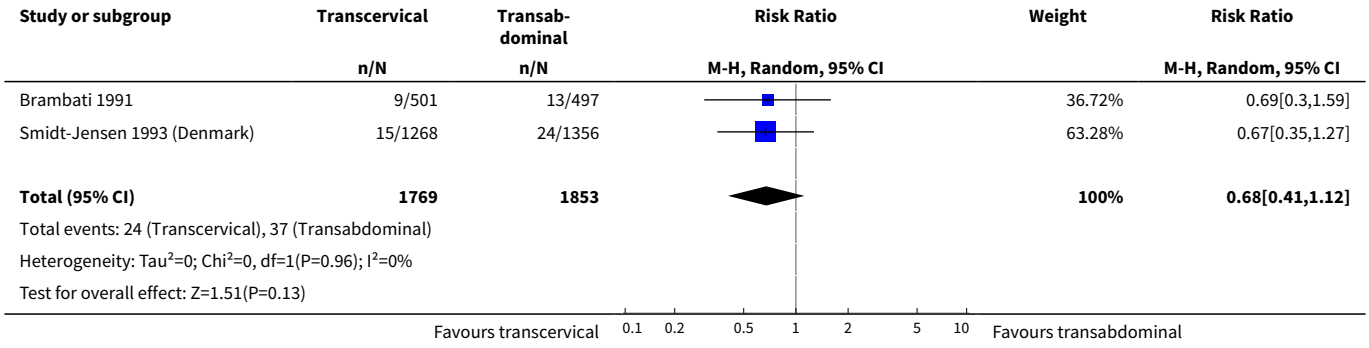
Analysis 4.16. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 16 Stillbirths.



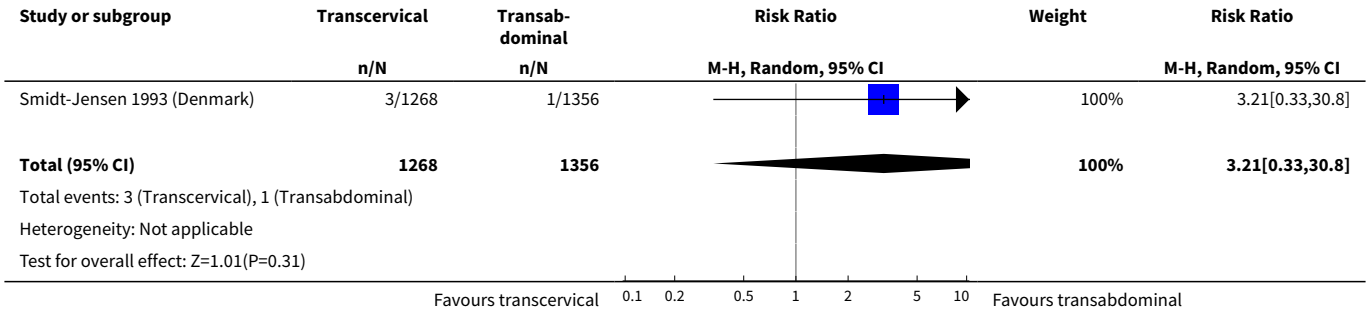
Analysis 4.17. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 17 Neonatal deaths.



Analysis 4.18. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 18 Anomalies (all recorded).



Analysis 4.19. Comparison 4 Transcervical versus transabdominal chorionic villus sampling (CVS), Outcome 19 Talipes.

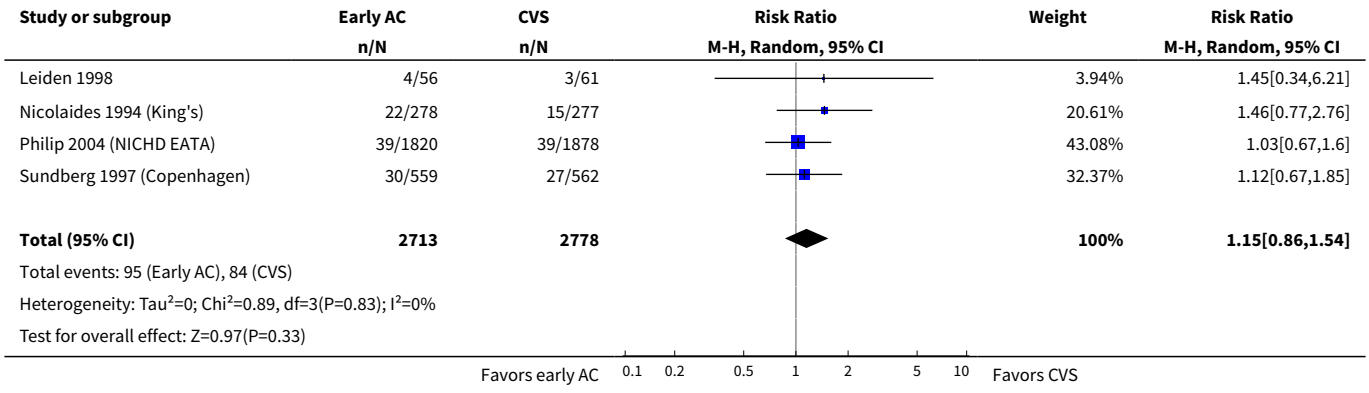


Comparison 5. Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All known pregnancy loss (including termination of pregnancy)	4	5491	Risk Ratio (M-H, Random, 95% CI)	1.15 [0.86, 1.54]
2 Spontaneous miscarriage	4	5491	Risk Ratio (M-H, Random, 95% CI)	1.73 [1.15, 2.60]
3 Spontaneous miscarriage after test	4	5489	Risk Ratio (M-H, Random, 95% CI)	1.71 [1.12, 2.61]
4 Non-compliance with allocated procedure	4	5566	Risk Ratio (M-H, Random, 95% CI)	0.25 [0.09, 0.72]
5 Sampling failure	4	5566	Risk Ratio (M-H, Random, 95% CI)	0.58 [0.24, 1.38]
6 Multiple insertions	3	4445	Risk Ratio (M-H, Random, 95% CI)	0.45 [0.21, 0.95]
7 Second test performed	4	5566	Risk Ratio (M-H, Random, 95% CI)	0.63 [0.28, 1.43]
8 Laboratory failure	4	5566	Risk Ratio (M-H, Random, 95% CI)	0.74 [0.34, 1.63]
9 All non-mosaic abnormalities	4	5566	Risk Ratio (M-H, Random, 95% CI)	0.95 [0.47, 1.90]
10 True mosaics	3	5451	Risk Ratio (M-H, Random, 95% CI)	0.47 [0.10, 2.20]
11 Confined mosaics	4	5566	Risk Ratio (M-H, Random, 95% CI)	0.82 [0.27, 2.47]
12 Maternal contamination	2	4330	Risk Ratio (M-H, Random, 95% CI)	1.92 [0.02, 162.80]
13 Known false positive after birth	2	670	Risk Ratio (M-H, Random, 95% CI)	0.36 [0.02, 8.73]
14 Knonw false negative after birth	1	555	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
15 Reporting time	1	3775	Mean Difference (IV, Random, 95% CI)	4.00 [3.82, 4.18]
16 Vaginal bleeding after 20 weeks	1	3698	Risk Ratio (M-H, Random, 95% CI)	0.71 [0.35, 1.43]
17 Amniotic leakage after test	3	4934	Risk Ratio (M-H, Random, 95% CI)	3.35 [0.37, 30.09]
18 Vaginal bleeding after test	3	4934	Risk Ratio (M-H, Random, 95% CI)	0.69 [0.42, 1.12]
19 Pre-labour ruptured membranes before 28 weeks	1	3698	Risk Ratio (M-H, Random, 95% CI)	0.50 [0.27, 0.92]
20 Delivery before 37 weeks	3	1755	Risk Ratio (M-H, Random, 95% CI)	1.16 [0.78, 1.74]
21 Delivery before 33 weeks	1	1121	Risk Ratio (M-H, Random, 95% CI)	0.50 [0.09, 2.73]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
22 Termination of pregnancy (all)	4	5489	Risk Ratio (M-H, Random, 95% CI)	0.74 [0.45, 1.25]
23 Perinatal deaths	4	5428	Risk Ratio (M-H, Random, 95% CI)	1.10 [0.53, 2.28]
24 Stillbirths	4	5428	Risk Ratio (M-H, Random, 95% CI)	1.11 [0.52, 2.36]
25 Neonatal deaths	4	5455	Risk Ratio (M-H, Random, 95% CI)	0.41 [0.05, 3.11]
26 All recorded deaths after viability	4	5453	Risk Ratio (M-H, Random, 95% CI)	1.18 [0.43, 3.24]
27 Anomalies (all recorded)	4	5305	Risk Ratio (M-H, Random, 95% CI)	1.14 [0.57, 2.30]
28 Talipes equinovarus	4	5305	Risk Ratio (M-H, Random, 95% CI)	3.75 [1.42, 9.88]
29 Haemangioma	4	5305	Risk Ratio (M-H, Random, 95% CI)	0.75 [0.26, 2.20]
30 Neonatal respiratory distress syndrome	4	4725	Risk Ratio (M-H, Random, 95% CI)	0.91 [0.21, 3.98]
31 Birthweight below 10th centile	1	3618	Risk Ratio (M-H, Random, 95% CI)	0.84 [0.66, 1.06]
32 Birthweight below 5th centile	2	629	Risk Ratio (M-H, Random, 95% CI)	0.66 [0.05, 9.38]

Analysis 5.1. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 1 All known pregnancy loss (including termination of pregnancy).



Analysis 5.2. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 2 Spontaneous miscarriage.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	4/56	3/61		7.97%	1.45[0.34,6.21]
Nicolaides 1994 (King's)	16/278	5/277		17.14%	3.19[1.18,8.58]
Philip 2004 (NICHD EATA)	22/1820	15/1878		39.41%	1.51[0.79,2.91]
Sundberg 1997 (Copenhagen)	20/559	13/562		35.48%	1.55[0.78,3.08]
Total (95% CI)	**2713**	**2778**		**100%**	**1.73[1.15,2.6]**
Total events: 62 (Early AC), 36 (CVS)					
Heterogeneity: Tau^2=0; Chi^2=1.79, df=3(P=0.62); I^2=0%					
Test for overall effect: Z=2.61(P=0.01)					

Analysis 5.3. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 3 Spontaneous miscarriage after test.

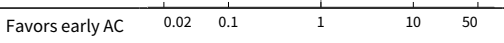
Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	3/55	2/60		5.85%	1.64[0.28,9.43]
Nicolaides 1994 (King's)	16/278	5/277		18.31%	3.19[1.18,8.58]
Philip 2004 (NICHD EATA)	22/1820	15/1878		42.1%	1.51[0.79,2.91]
Sundberg 1997 (Copenhagen)	17/559	12/562		33.73%	1.42[0.69,2.95]
Total (95% CI)	**2712**	**2777**		**100%**	**1.71[1.12,2.61]**
Total events: 58 (Early AC), 34 (CVS)					
Heterogeneity: Tau^2=0; Chi^2=1.91, df=3(P=0.59); I^2=0%					
Test for overall effect: Z=2.47(P=0.01)					

Analysis 5.4. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 4 Non-compliance with allocated procedure.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	1/55	10/60		26.87%	0.11[0.01,0.82]
Nicolaides 1994 (King's)	0/278	0/277			Not estimable
Philip 2004 (NICHD EATA)	3/1861	7/1914		60.23%	0.44[0.11,1.7]
Sundberg 1997 (Copenhagen)	0/559	4/562		12.9%	0.11[0.01,2.07]
Total (95% CI)	**2753**	**2813**		**100%**	**0.25[0.09,0.72]**
Total events: 4 (Early AC), 21 (CVS)					
Heterogeneity: Tau^2=0; Chi^2=1.69, df=2(P=0.43); I^2=0%					
Test for overall effect: Z=2.56(P=0.01)					

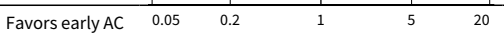
Analysis 5.5. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 5 Sampling failure.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	1/55	2/60		12%	0.55[0.05,5.85]
Nicolaides 1994 (King's)	0/278	2/277		7.67%	0.2[0.01,4.13]
Philip 2004 (NICHD EATA)	9/1861	8/1914		47.5%	1.16[0.45,2.99]
Sundberg 1997 (Copenhagen)	3/559	11/562		32.83%	0.27[0.08,0.98]
Total (95% CI)	**2753**	**2813**		**100%**	**0.58[0.24,1.38]**
Total events: 13 (Early AC), 23 (CVS)					
Heterogeneity: Tau^2=0.18; Chi^2=3.83, df=3(P=0.28); I^2=21.6%					
Test for overall effect: Z=1.24(P=0.21)					



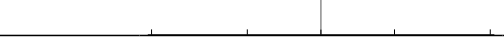
Analysis 5.6. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 6 Multiple insertions.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	5/55	25/60		33.23%	0.22[0.09,0.53]
Nicolaides 1994 (King's)	4/278	7/277		23.61%	0.57[0.17,1.92]
Philip 2004 (NICHD EATA)	16/1861	24/1914		43.17%	0.69[0.37,1.29]
Total (95% CI)	**2194**	**2251**		**100%**	**0.45[0.21,0.95]**
Total events: 25 (Early AC), 56 (CVS)					
Heterogeneity: Tau^2=0.24; Chi^2=4.36, df=2(P=0.11); I^2=54.1%					
Test for overall effect: Z=2.09(P=0.04)					



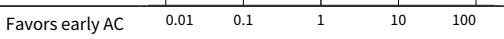
Analysis 5.7. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 7 Second test performed.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	4/55	4/60		22.34%	1.09[0.29,4.15]
Nicolaides 1994 (King's)	5/278	7/277		26.76%	0.71[0.23,2.22]
Philip 2004 (NICHD EATA)	13/1861	16/1914		38.31%	0.84[0.4,1.73]
Sundberg 1997 (Copenhagen)	1/559	13/562		12.58%	0.08[0.01,0.59]
Total (95% CI)	**2753**	**2813**		**100%**	**0.63[0.28,1.43]**
Total events: 23 (Early AC), 40 (CVS)					
Heterogeneity: Tau^2=0.32; Chi^2=5.64, df=3(P=0.13); I^2=46.81%					
Test for overall effect: Z=1.1(P=0.27)					



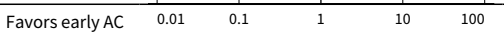
Analysis 5.8. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 8 Laboratory failure.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	1/55	0/60		6.13%	3.27[0.14,78.58]
Nicolaides 1994 (King's)	5/278	7/277		48.06%	0.71[0.23,2.22]
Philip 2004 (NICHD EATA)	4/1861	4/1914		32.33%	1.03[0.26,4.11]
Sundberg 1997 (Copenhagen)	1/559	5/562		13.48%	0.2[0.02,1.72]
Total (95% CI)	**2753**	**2813**		**100%**	**0.74[0.34,1.63]**
Total events: 11 (Early AC), 16 (CVS)					
Heterogeneity: Tau^2=0; Chi^2=2.49, df=3(P=0.48); I^2=0%					
Test for overall effect: Z=0.74(P=0.46)					



Analysis 5.9. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 9 All non-mosaic abnormalities.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	2/55	0/60		4.91%	5.45[0.27,111.01]
Nicolaides 1994 (King's)	5/278	5/277		21.45%	1[0.29,3.4]
Philip 2004 (NICHD EATA)	37/1861	31/1914		49.95%	1.23[0.76,1.97]
Sundberg 1997 (Copenhagen)	4/559	11/562		23.69%	0.37[0.12,1.14]
Total (95% CI)	**2753**	**2813**		**100%**	**0.95[0.47,1.9]**
Total events: 48 (Early AC), 47 (CVS)					
Heterogeneity: Tau^2=0.19; Chi^2=4.87, df=3(P=0.18); I^2=38.44%					
Test for overall effect: Z=0.15(P=0.88)					

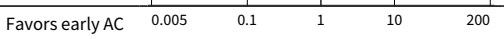


Analysis 5.10. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 10 True mosaics.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Nicolaides 1994 (King's)	0/278	1/277		23.1%	0.33[0.01,8.12]
Philip 2004 (NICHD EATA)	1/1861	3/1914		46.12%	0.34[0.04,3.29]
Sundberg 1997 (Copenhagen)	1/559	1/562		30.78%	1.01[0.06,16.03]
Total (95% CI)	**2698**	**2753**		**100%**	**0.47[0.1,2.2]**
Total events: 2 (Early AC), 5 (CVS)					
Heterogeneity: Tau^2=0; Chi^2=0.41, df=2(P=0.81); I^2=0%					
Test for overall effect: Z=0.95(P=0.34)					

Analysis 5.11. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 11 Confined mosaics.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	2/55	0/60		11.47%	5.45[0.27,111.01]
Nicolaides 1994 (King's)	0/278	2/277		11.36%	0.2[0.01,4.13]
Philip 2004 (NICHD EATA)	24/1861	24/1914		65.34%	1.03[0.59,1.8]
Sundberg 1997 (Copenhagen)	0/559	3/562		11.83%	0.14[0.01,2.77]
Total (95% CI)	**2753**	**2813**		**100%**	**0.82[0.27,2.47]**
Total events: 26 (Early AC), 29 (CVS)					
Heterogeneity: Tau^2=0.4; Chi^2=3.97, df=3(P=0.26); I^2=24.39%					
Test for overall effect: Z=0.35(P=0.72)					



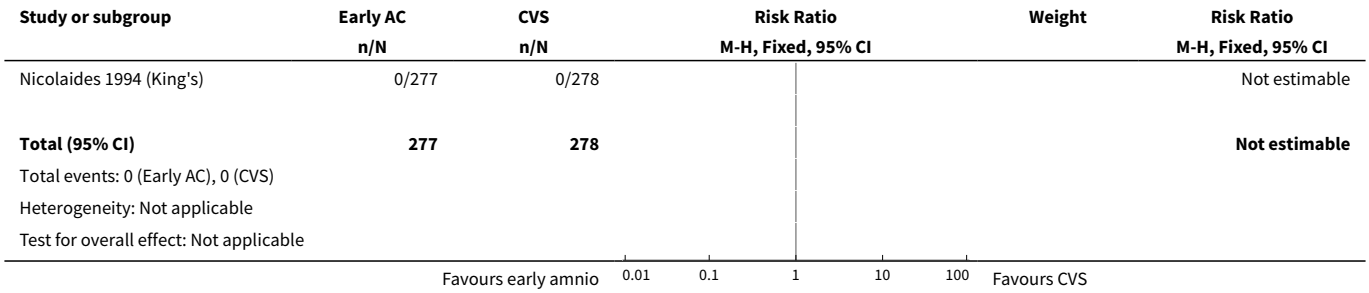
Analysis 5.12. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 12 Maternal contamination.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Nicolaides 1994 (King's)	0/278	2/277		49.33%	0.2[0.01,4.13]
Philip 2004 (NICHD EATA)	8/1861	0/1914		50.67%	17.48[1.01,302.7]
Total (95% CI)	**2139**	**2191**		**100%**	**1.92[0.02,162.8]**
Total events: 8 (Early AC), 2 (CVS)					
Heterogeneity: Tau^2=8; Chi^2=4.55, df=1(P=0.03); I^2=78.02%					
Test for overall effect: Z=0.29(P=0.77)					

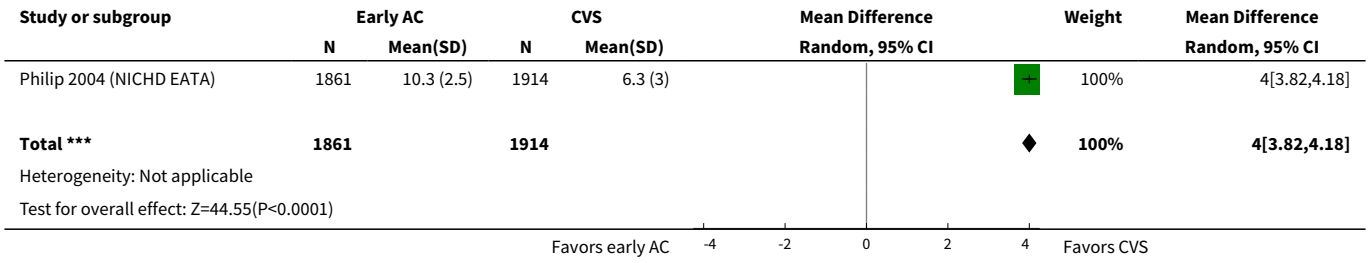
Analysis 5.13. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 13 Known false positive after birth.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	0/55	1/60		100%	0.36[0.02,8.73]
Nicolaides 1994 (King's)	0/278	0/277			Not estimable
Total (95% CI)	**333**	**337**		**100%**	**0.36[0.02,8.73]**
Total events: 0 (Early AC), 1 (CVS)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.62(P=0.53)					

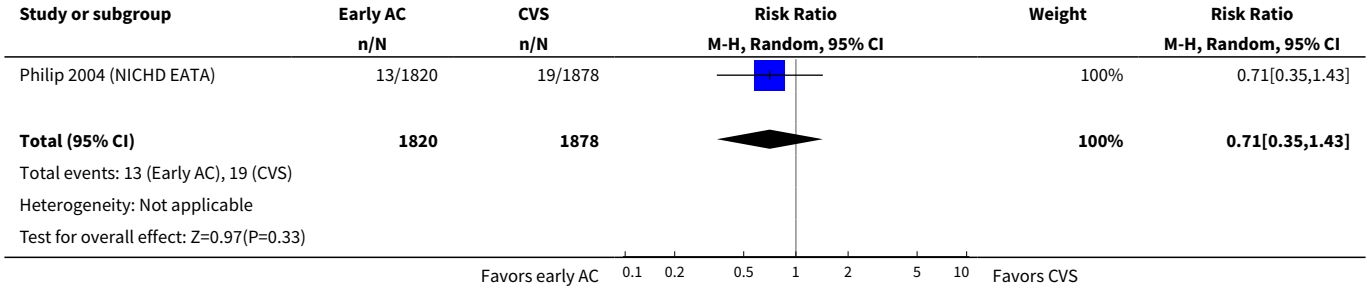
Analysis 5.14. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 14 Knonw false negative after birth.



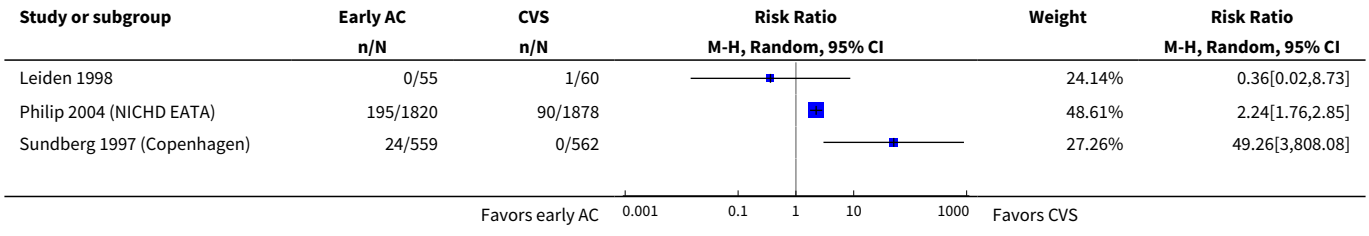
Analysis 5.15. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 15 Reporting time.

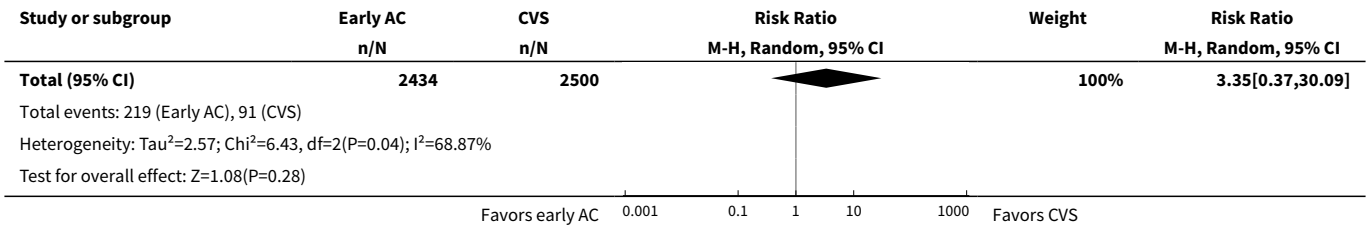


Analysis 5.16. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 16 Vaginal bleeding after 20 weeks.

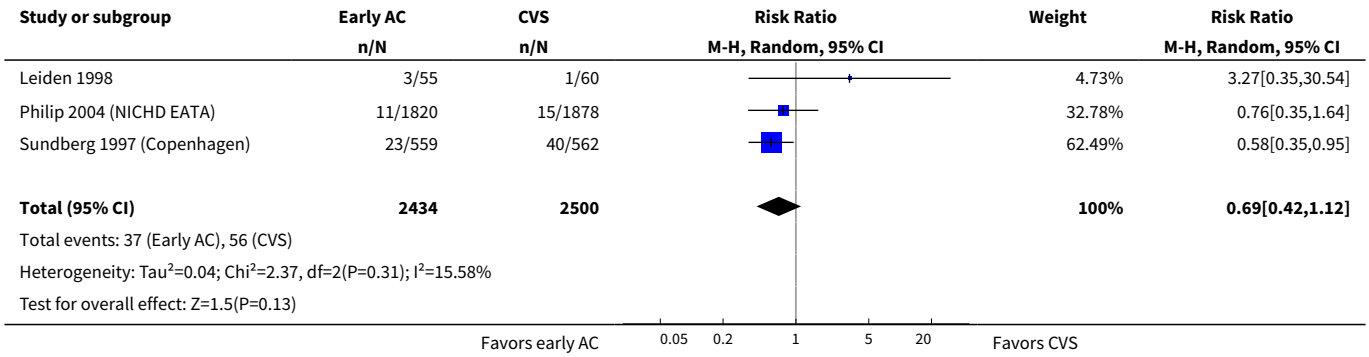


Analysis 5.17. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 17 Amniotic leakage after test.

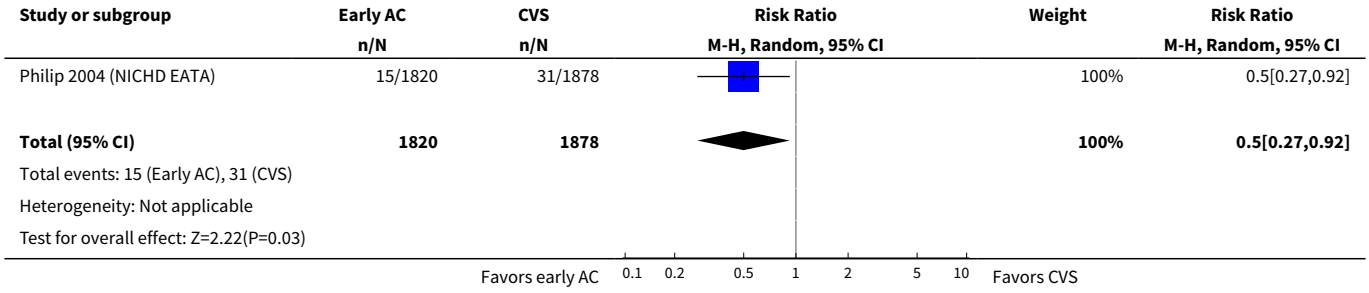




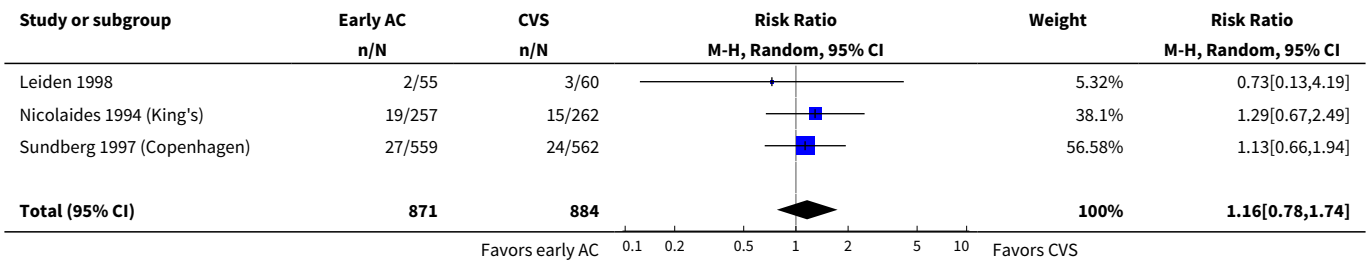
Analysis 5.18. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 18 Vaginal bleeding after test.

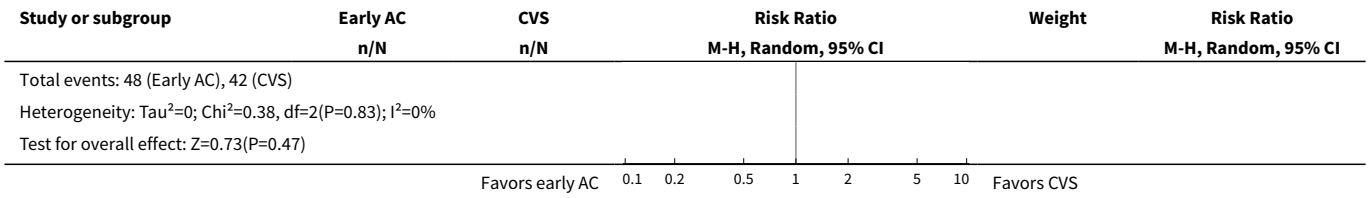


Analysis 5.19. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 19 Pre-labour ruptured membranes before 28 weeks.

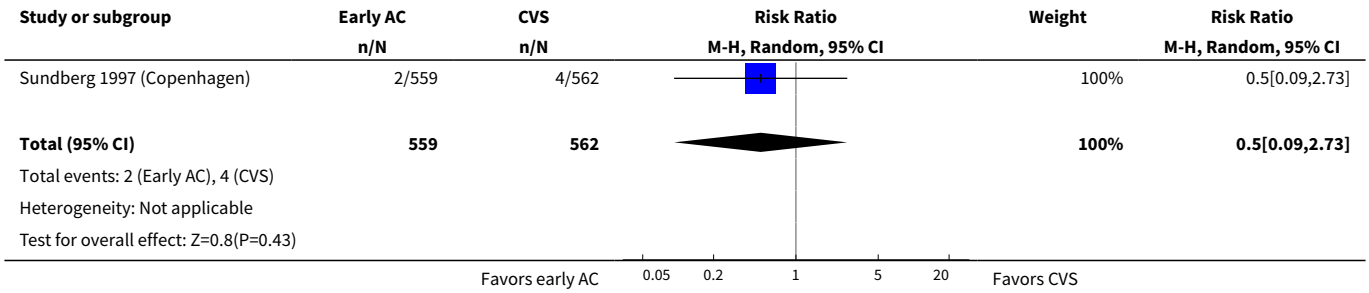


Analysis 5.20. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 20 Delivery before 37 weeks.

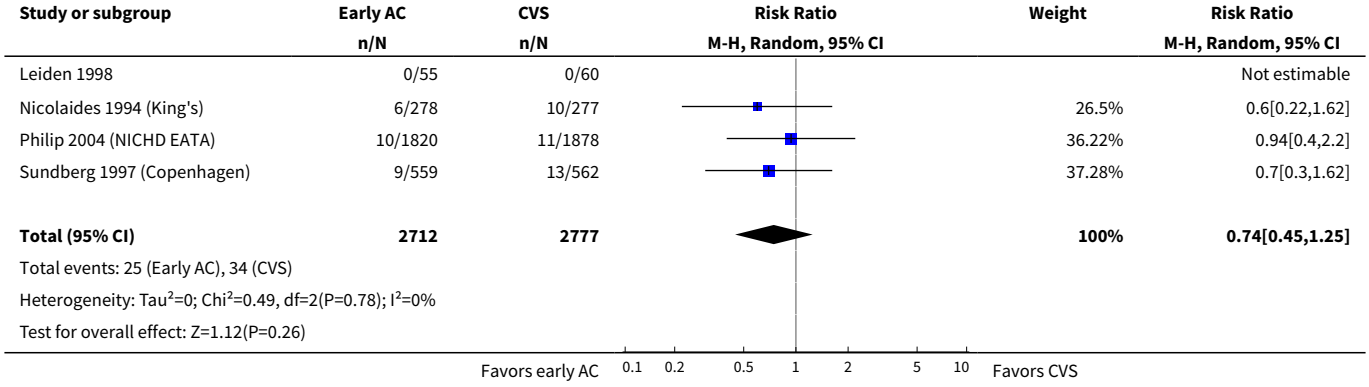




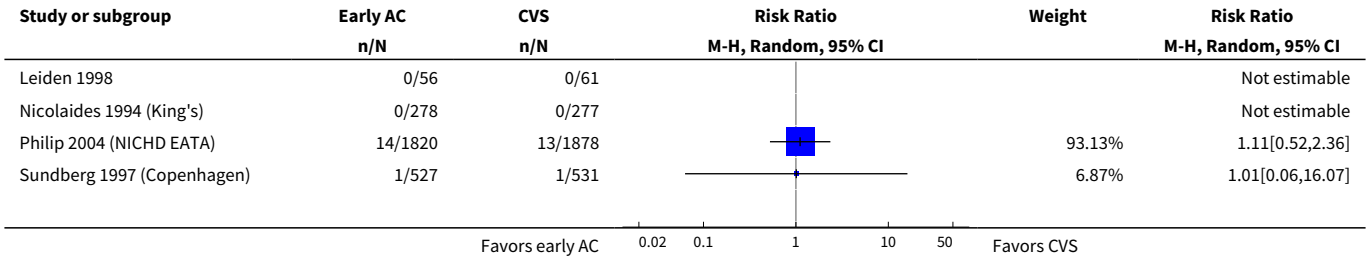
Analysis 5.21. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 21 Delivery before 33 weeks.

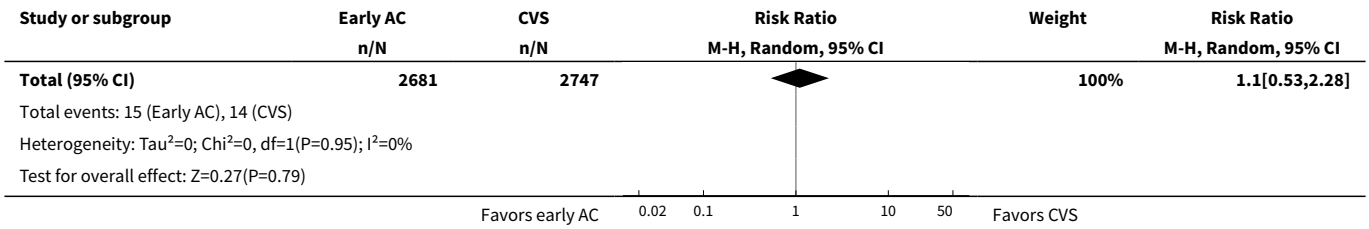


Analysis 5.22. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 22 Termination of pregnancy (all).

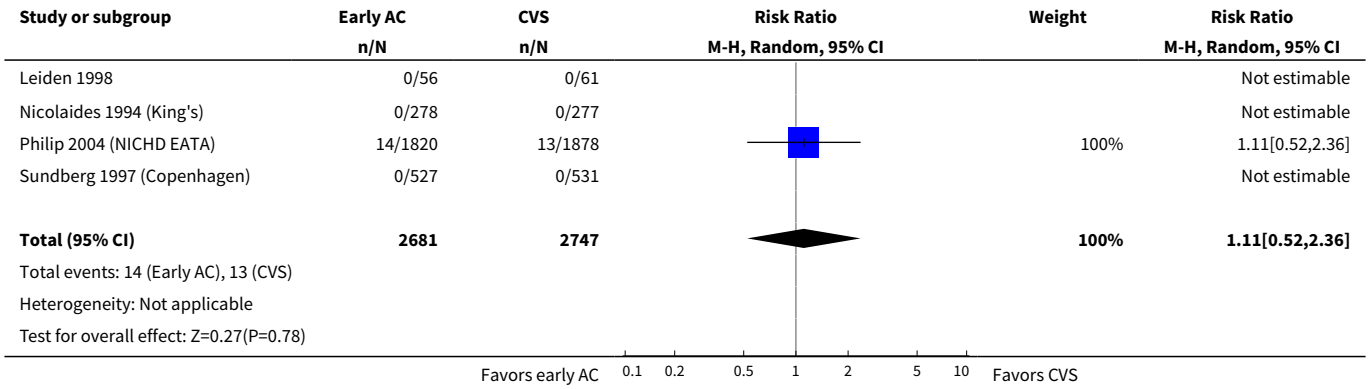


Analysis 5.23. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 23 Perinatal deaths.

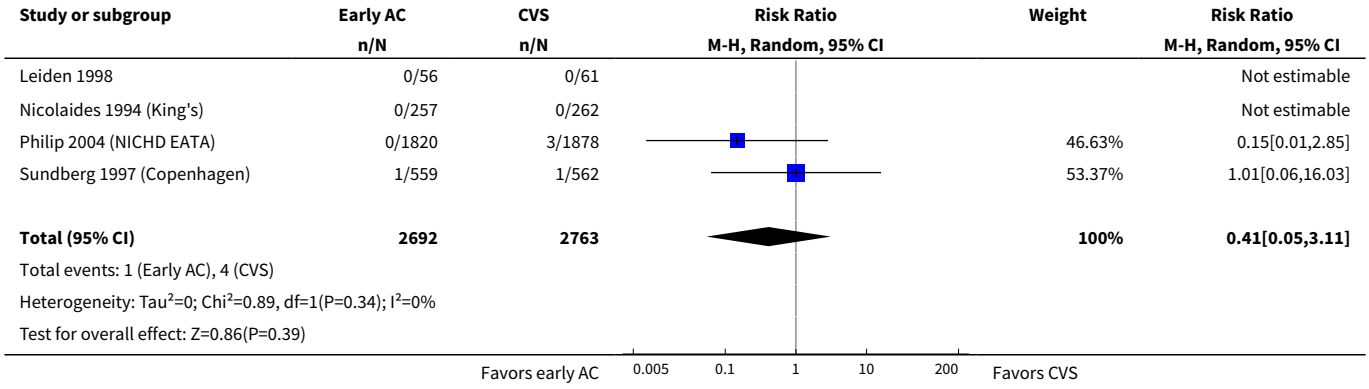




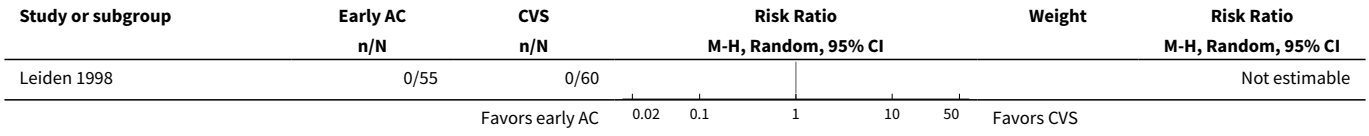
Analysis 5.24. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 24 Stillbirths.

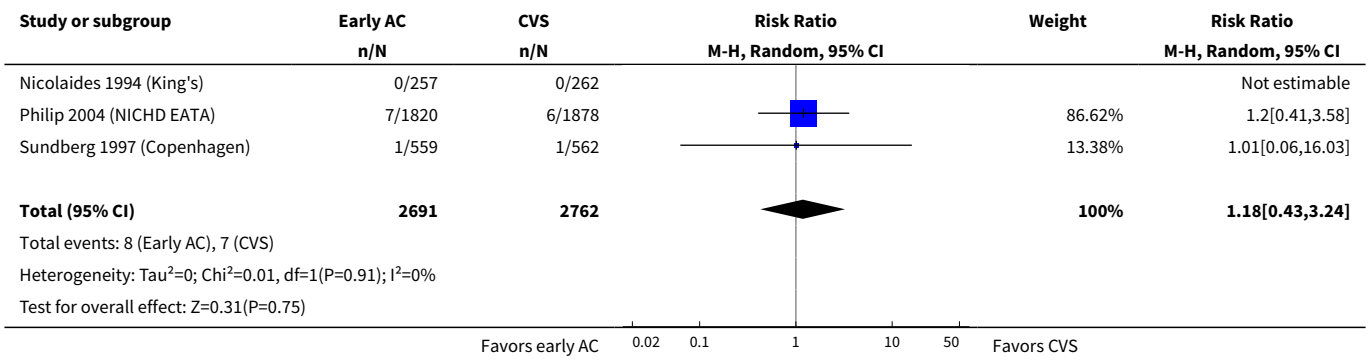


Analysis 5.25. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 25 Neonatal deaths.

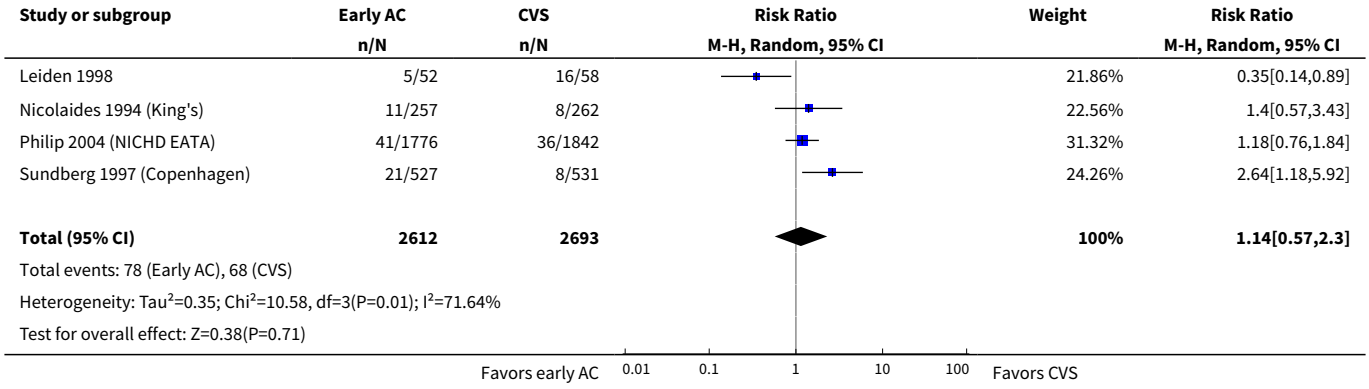


Analysis 5.26. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 26 All recorded deaths after viability.

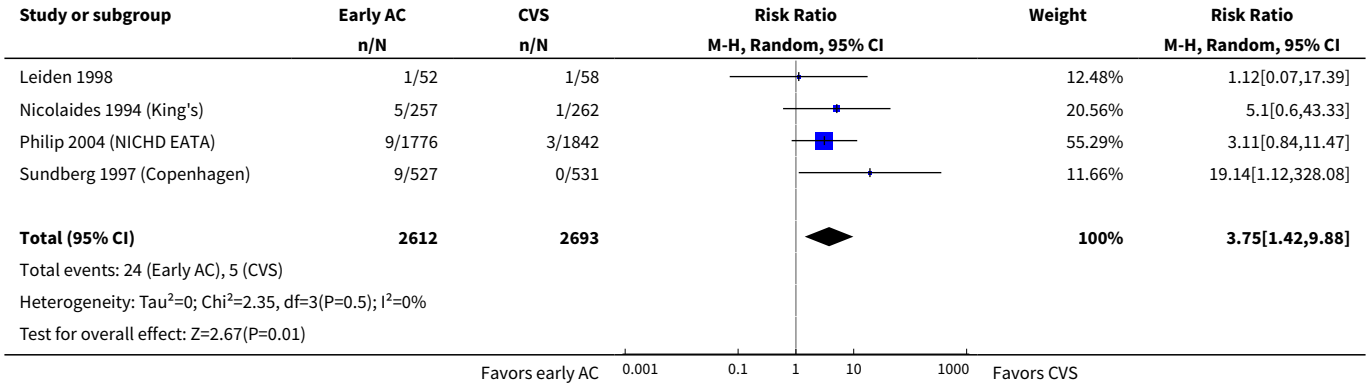




Analysis 5.27. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 27 Anomalies (all recorded).

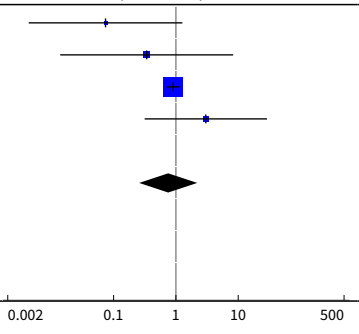


Analysis 5.28. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 28 Talipes equinovarus.



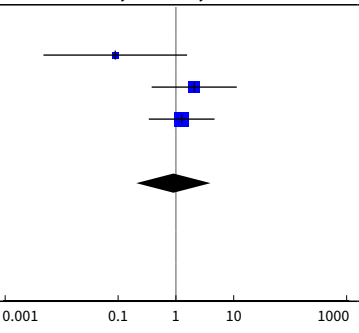
Analysis 5.29. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 29 Haemangioma.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	0/52	7/58		11.7%	0.07[0,1.27]
Nicolaides 1994 (King's)	0/257	1/262		9.6%	0.34[0.01,8.3]
Philip 2004 (NICHD EATA)	115/1776	132/1842		62.02%	0.9[0.71,1.15]
Sundberg 1997 (Copenhagen)	3/527	1/531		16.69%	3.02[0.32,28.97]
Total (95% CI)	**2612**	**2693**		**100%**	**0.75[0.26,2.2]**
Total events: 118 (Early AC), 141 (CVS)					
Heterogeneity: Tau^2=0.47; Chi^2=4.48, df=3(P=0.21); I^2=33.04%					
Test for overall effect: Z=0.52(P=0.6)					



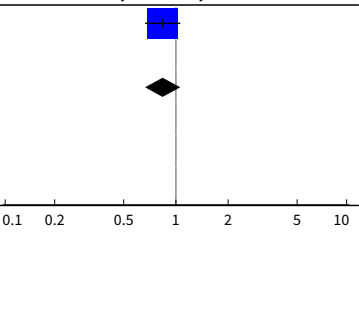
Analysis 5.30. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 30 Neonatal respiratory distress syndrome.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Leiden 1998	0/52	0/58			Not estimable
Nicolaides 1994 (King's)	0/74	6/86		19.27%	0.09[0.01,1.56]
Philip 2004 (NICHD EATA)	4/1667	2/1730		36.09%	2.08[0.38,11.32]
Sundberg 1997 (Copenhagen)	5/527	4/531		44.64%	1.26[0.34,4.66]
Total (95% CI)	**2320**	**2405**		**100%**	**0.91[0.21,3.98]**
Total events: 9 (Early AC), 12 (CVS)					
Heterogeneity: Tau^2=0.83; Chi^2=3.92, df=2(P=0.14); I^2=48.98%					
Test for overall effect: Z=0.13(P=0.9)					

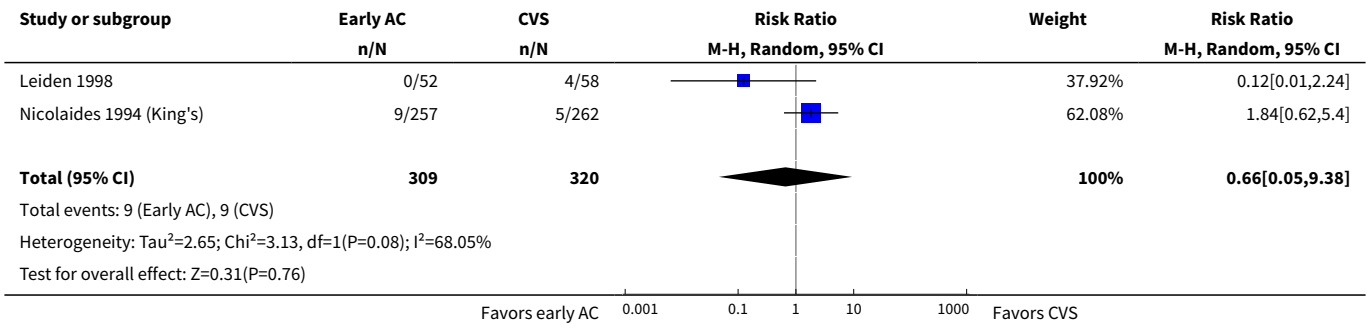


Analysis 5.31. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 31 Birthweight below 10th centile.

Study or subgroup	Early AC n/N	CVS n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Philip 2004 (NICHD EATA)	118/1776	146/1842		100%	0.84[0.66,1.06]
Total (95% CI)	**1776**	**1842**		**100%**	**0.84[0.66,1.06]**
Total events: 118 (Early AC), 146 (CVS)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.48(P=0.14)					



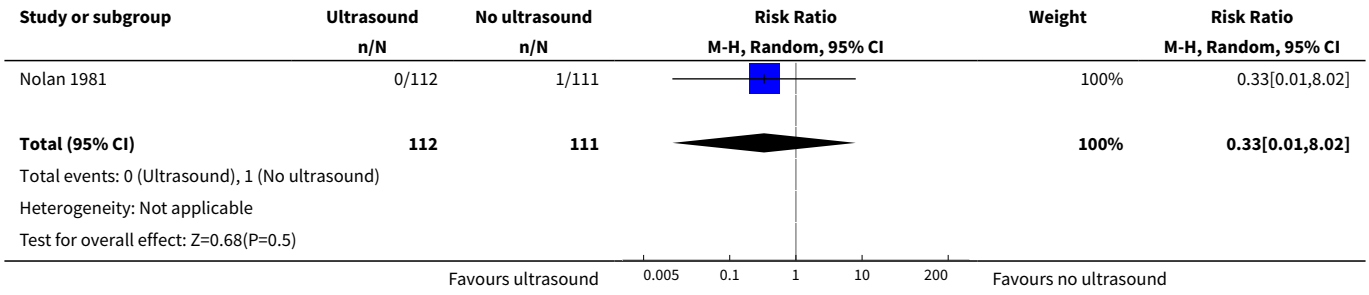
Analysis 5.32. Comparison 5 Early amniocentesis (AC) versus transabdominal chorionic villus sampling (CVS), Outcome 32 Birthweight below 5th centile.



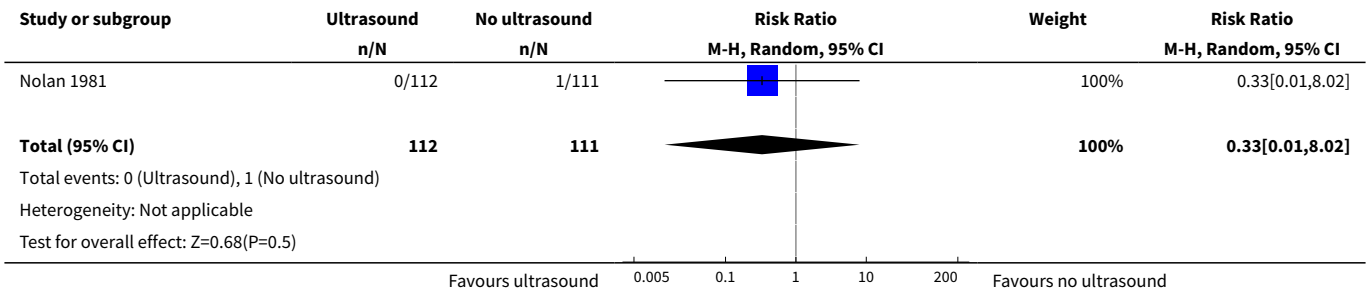
Comparison 6. Ultrasound versus no ultrasound before second trimester amniocentesis

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All known pregnancy loss (including termination of pregnancy)	1	223	Risk Ratio (M-H, Random, 95% CI)	0.33 [0.01, 8.02]
2 Spontaneous miscarriage	1	223	Risk Ratio (M-H, Random, 95% CI)	0.33 [0.01, 8.02]
3 Spontaneous miscarriage after test	1	223	Risk Ratio (M-H, Random, 95% CI)	0.33 [0.01, 8.02]
4 Sampling failure	1	223	Risk Ratio (M-H, Random, 95% CI)	10.90 [0.61, 194.85]
5 Multiple insertions	1	223	Risk Ratio (M-H, Random, 95% CI)	0.67 [0.41, 1.09]
6 Bloody tap (not pre-specified)	1	223	Risk Ratio (M-H, Random, 95% CI)	1.87 [0.87, 4.02]

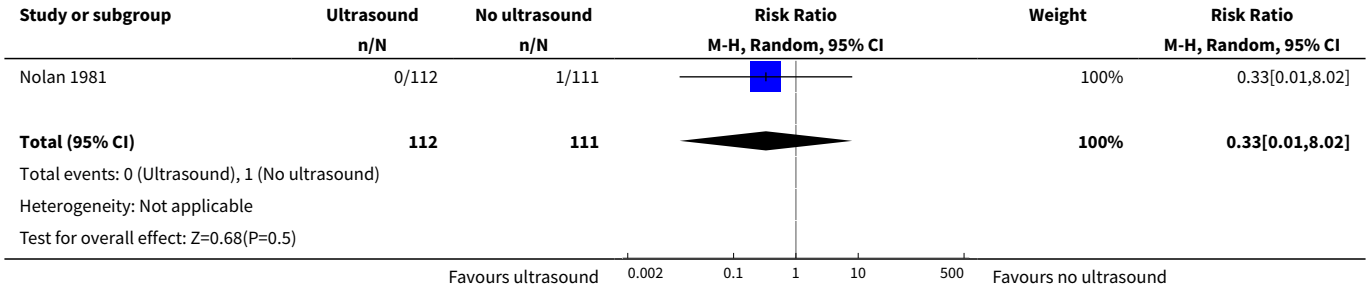
Analysis 6.1. Comparison 6 Ultrasound versus no ultrasound before second trimester amniocentesis, Outcome 1 All known pregnancy loss (including termination of pregnancy).



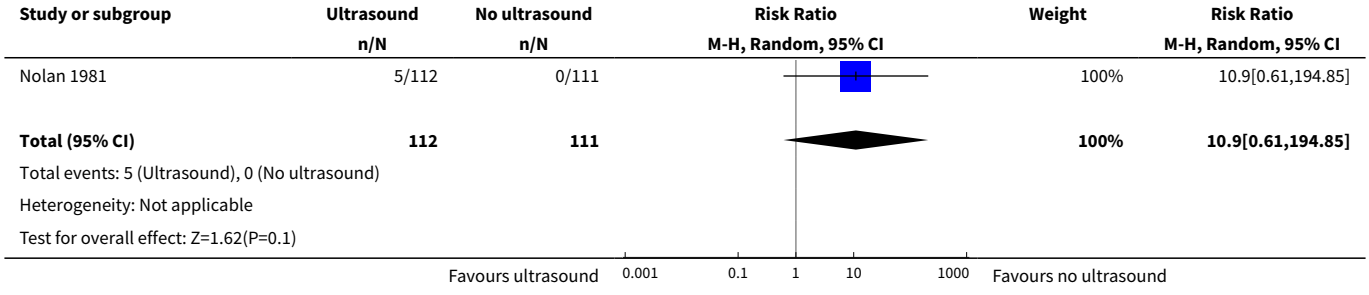
Analysis 6.2. Comparison 6 Ultrasound versus no ultrasound before second trimester amniocentesis, Outcome 2 Spontaneous miscarriage.



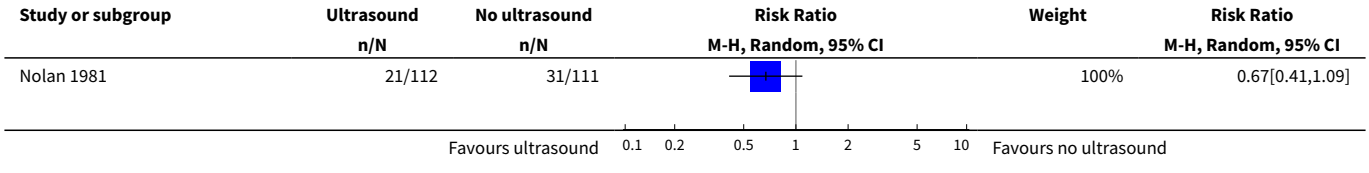
Analysis 6.3. Comparison 6 Ultrasound versus no ultrasound before second trimester amniocentesis, Outcome 3 Spontaneous miscarriage after test.

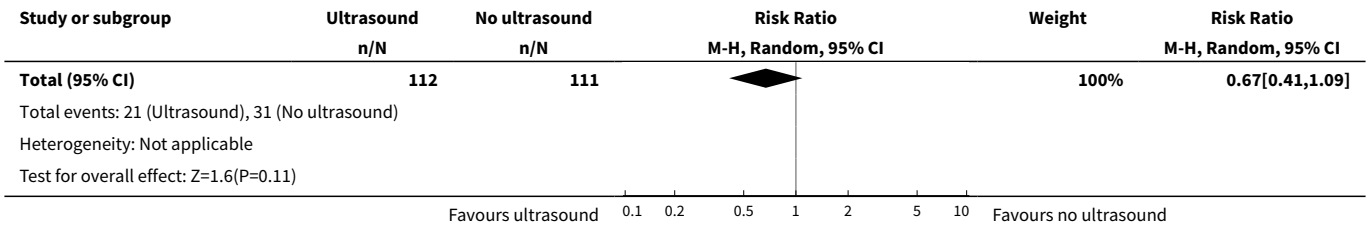


Analysis 6.4. Comparison 6 Ultrasound versus no ultrasound before second trimester amniocentesis, Outcome 4 Sampling failure.

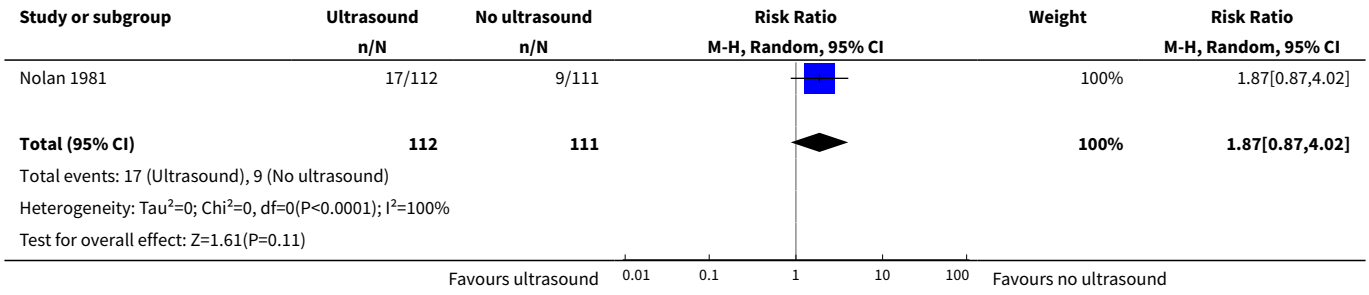


Analysis 6.5. Comparison 6 Ultrasound versus no ultrasound before second trimester amniocentesis, Outcome 5 Multiple insertions.





Analysis 6.6. Comparison 6 Ultrasound versus no ultrasound before second trimester amniocentesis, Outcome 6 Bloody tap (not pre-specified).



ADDITIONAL TABLES

Table 1. Systematic reviews of observational studies (chorionic villus sampling)

Systematic review (by first author)	Single or multiple pregnancies	Pregnancy loss rates reported % (95% CI)	Pooled procedure-related pregnancy losses % (95% CI)
Agarwal 2012	Twins	Total 3.84% (2.48 to 5.47) < 20 weeks 2.75% (1.28 to 4.75) < 28 weeks 3.44% (1.67 to 5.81)	-
Akolekar 2015	Singletons	< 24 weeks 2.18% (1.61 to 2.82)	0.22% (-0.71 to 1.16)
Mujezinovic 2007	Singletons	Total 2.0% (1.0 to 1.7) < 24 weeks 1.3% (0.5 to 2.3)	< 14 days 0.7% (0.3 to 1.4)

Table 2. Systematic reviews of observational studies (amniocentesis)

Systematic review (by first author)	Single or multiple pregnancies	Pregnancy loss rates reported % (95% CI)	Pooled procedure-related pregnancy losses % (95% CI)
Agarwal 2012	Twins	Total 3.07% (1.83 to 4.61)	-

Table 2. Systematic reviews of observational studies (amniocentesis) *(Continued)*

		< 20 weeks 2.25% (1.23 to 3.57) < 24 weeks 2.54% (1.43 to 3.96) < 28 weeks 1.70% (0.37 to 3.97)	
Akolekar 2015	Singletons	< 24 weeks 0.81% (0.58 to 1.08)	0.11% (-0.04 to 0.26)
Mujezinovic 2007	Singletons	Total 1.9% (1.4 to 2.5) < 24 weeks 0.9% (0.60 to 1.30)	< 14 days 0.6 (0.5-0.7)
Vink 2012	Twins	Monochorionic 7.7% vs 1.4% (amniocentesis vs control)	< 24 weeks 3.5% (2.6 to 4.7)

APPENDICES

Appendix 1. Search terms used in ClinicalTrials.gov and WHO ICTRP

amniocentesis AND CVS

amniocentesis AND chorionic

WHAT'S NEW

Date	Event	Description
3 March 2017	New search has been performed	Search updated and one new trial, ISRCTN18010960, identified and excluded from this update. Five 'Summary of findings' tables incorporated. Primary and secondary outcomes specified.
3 March 2017	New citation required but conclusions have not changed	New citations identified. One trial excluded and additional reports added for already included studies Borrell 1999, Canada 1989 and Philip 2004 (NICHD EATA).

HISTORY

Protocol first published: Issue 3, 2001
Review first published: Issue 3, 2003

Date	Event	Description
3 January 2009	Amended	Minor edits to the conclusions, and information added to the Notes section of Borrell 1999.
29 June 2008	New search has been performed	New included study added (Philip 2004 (NICHD EATA)). Other minor amendments made including updating the reference list. There are no significant changes to the conclusions.
29 December 2007	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

Z Alfirevic developed the protocol, interpreted the data, and wrote the original review. F Mujezinovic extracted the data and co-wrote the original review. K Navaratnam completed the GRADE assessments for included studies and updated the review.

DECLARATIONS OF INTEREST

Zarko Alfirevic: Zarko Alfirevic is Director of the Harris Wellbeing Preterm Birth Centre, which is grant funded by the charity Wellbeing of Women. This grant is administered by the University of Liverpool, and Zarko Alfirevic is not paid directly. He is the principal investigator or co-investigator on several grants from public funders, including National Institute of Health Research, British Medical Association, European Commission, and WHO. He has received research support in the past from Perkin Elmer and Alere for research related to pre-eclampsia and preterm birth prevention. These grants were administered by his employers and ZA did not benefit directly. ZA is also a Co-coordinating Editor of Cochrane Pregnancy and Childbirth.

Kate Navaratnam: none known.

Faris Mujezinovic: none known.

SOURCES OF SUPPORT

Internal sources

- The University of Liverpool, UK.

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Methods updated to the current standard methods text for Cochrane Pregnancy and Childbirth. Searches of ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform (ICTRP) were added.

Five 'Summary of findings' tables were incorporated. Primary and secondary outcomes were specified.

The following outcomes were not pre-specified in the protocol:

- Results given in less than 7 days (not pre-specified)
- Results given in less than 14 days (not pre-specified)
- Results given in less than 21 days (not pre-specified)
- Results given after 21 days (not pre-specified)
- Not wanting another baby at 22 weeks' gestation (not pre-specified)

INDEX TERMS

Medical Subject Headings (MeSH)

Amniocentesis [*adverse effects] [standards]; Chorionic Villi Sampling [*adverse effects] [standards]; Congenital Abnormalities [diagnosis]; Pregnancy Trimester, First; Pregnancy Trimester, Second; Randomized Controlled Trials as Topic

MeSH check words

Female; Humans; Pregnancy